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STUDIES IN NUTRITION.

I. THE NUTRITIVE VALUE OF COCONUT GLOBULIN AND COCONUT PRESS CAKE.

By CARL O. JOHNS, A. J. FINKS, and MABEL S. PAUL.

(From the Protein Investigation Laboratory, Bureau of Chemistry, Department of Agriculture, Washington.)

(Received for publication, February 7, 1919.)

The use of coconut products has increased rapidly in the United States during the last 3 years. Statistics obtained from the Department of Commerce show that during the three 12 month periods ending June 30, in the years 1916, 1917, and 1918, the imports of coconut products were valued at approximately $8,000,000, $25,000,000, and $63,000,000. During the 12 month period ending June 30, 1918, 486,996,112 pounds of copra or dried coconut meats, valued at $26,945,569, were imported. This is more than four times the quantity imported in 1916.

Copra is pressed in the oil mills of this country in order to obtain coconut oil, which is used to a great extent in the manufacture of vegetable oleomargarine, frequently called nut margarine. After pressing, a cake containing about 10 per cent of oil and 17 to 18 per cent of protein (N × 5.7) remains. In calculating the percentage of protein from the nitrogen content of coconut press cake, the factor 5.7 gives more accurately the percentage of protein than does the factor 6.25, since coconut globulin, which is the chief protein in coconut press cake, contains approximately 17.5 per cent of nitrogen. This is the average percentage of nitrogen found in ten different preparations of coconut globulin.

Coconut press cake is rapidly gaining favor in the United States as a cattle food. It has been found especially valuable when used as a protein concentrate in feeding milch cows. Experiments made both in the United States and in Europe show that its use tends to produce a milk with a high fat content.

The basic nitrogen of coconut globulin constitutes more than one-third of the total nitrogen, and the percentages of amino-acids in the globulin are as follows:[1]

Amino-Acid.	*per cent*
Cystine	1.44
Arginine	15.92
Histidine	2.42
Lysine	5.80

This globulin, therefore, contains all of the basic amino-acids which are known to be essential for normal growth. It also contains tryptophane. Compared with other vegetable proteins it contains a relatively high percentage of lysine which is present in but small quantities in the proteins of most cereal grains. It could be predicted that the protein of the coconut would therefore be of high quality. Nutrition experiments fully corroborate the chemical analyses. The isolated globulin, as well as the press cake, was fed as the sole source of protein to white rats, and normal growth was obtained, showing that the protein has a high biological value.

In the first experiment the rats were fed on a diet of isolated coconut globulin, together with the other essential ingredients of an adequate diet. The necessary salts and water-soluble vitamines were supplied by protein-free milk. This diet was eaten readily, and the rats showed none of the symptoms that accompany a deficient diet. The rate of growth was above normal and Rat 63 gave birth to eight young. The failure to rear them was probably due to the fact that the percentage of protein-free milk in the diet, while sufficient for normal growth of the mother, did not furnish enough water-soluble vitamine for both the mother and young. The curves in Chart I show the high biological value of coconut globulin.

Another experiment with coconut globulin was made, in which the salts were supplied by an artificial mixture approximating in composition the inorganic constituents of milk. The water-soluble vitamine in the diet was supplied by 2 per cent of dried brewers' yeast. As shown on Chart II, the rats grew at an almost

[1] Johns, C. O., Finks, A. J., and Gersdorff, C. E. F., *J. Biol. Chem.*, 1919, xxxvii, 149.

normal rate. The 2 per cent of yeast probably supplied scarcely enough water-soluble vitamine for normal growth. It was considered advisable, however, not to increase the percentage of yeast in the diet, in order to eliminate the possibility of adding

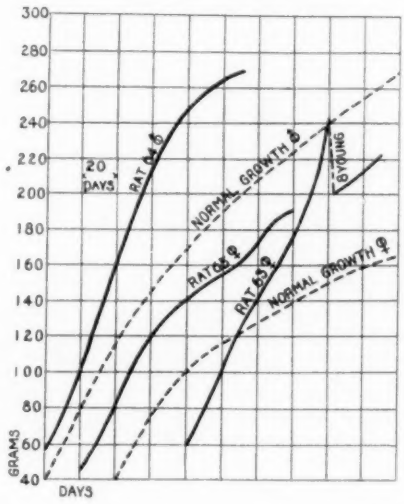


CHART I. Growth on a diet containing coconut globulin as the sole source of protein. More than average normal growth was obtained in each case. Rat 63 ♀ grew more rapidly than the normal rate of growth for a male rat. It was mated with Rat 64 ♂ when about 120 gm. in weight and gave birth to a litter of eight young which were not reared. The composition of the diet was as follows:

	per cent
Coconut globulin*	18
Protein-free milk	28
Agar	5
Starch	21
Butter fat	18
Lard	7

* Johns, C. O., Finks, A. J., and Gersdorff, C. E. F., *J. Biol. Chem.*, 1919, xxxvii, 149.

enough yeast protein to exert a supplementing effect on the coconut globulin.

In the third experiment commercial coconut press cake was used as the sole source of protein. The press cake, which was

ground to a meal, constituted 75 per cent of the diet, the remainder being 21 per cent of butter fat, and 4 per cent of an adequate salt mixture. This diet contained only 13.1 per cent

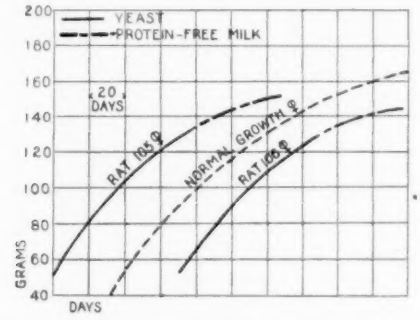


CHART II. Growth on coconut globulin, using 2 per cent of dried brewers' yeast as the source of water-soluble vitamine, and an artificial salt mixture replacing protein-free milk. The rate of growth was below that obtained when protein-free milk was used in the diet (Chart I). The supply of water-soluble vitamine furnished by 2 per cent of yeast was probably scarcely enough for normal growth. The composition of the diet was as follows:

	per cent
Coconut globulin	18
Salt mixture*	4
Dried brewers' yeast	2
Agar	5
Starch	46
Butter fat	18
Lard	7

*The composition of salt mixture is as follows (Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1917, xxxii, 374):

	gm.		*gm.*
$CaCO_3$	134.8	Citric acid + H_2O	111.1
$MgCO_3$	24.2	Fe citrate + $1\frac{1}{2}$ H_2O	6.34
Na_2CO_3	34.2	KI	0.020
K_2CO_3	141.3	$MnSO_4$	0.079
H_3PO_4	103.2	NaF	0.248
HCl	53.4	KAl $(SO_4)_2$	0.0245
H_2SO_4	9.2		

of protein (N × 5.7). Notwithstanding the relatively low protein content of this diet, several of the rats attained normal growth (Chart III). This experiment shows conclusively the

high biological value of coconut press cake. Because of its bulky character, the diet was far from ideal for nutrition experiments with rats, and would probably be more satisfactory for animals like cattle which have a large digestive tract and are accustomed to coarse, bulky diets. The growth obtained on this diet shows

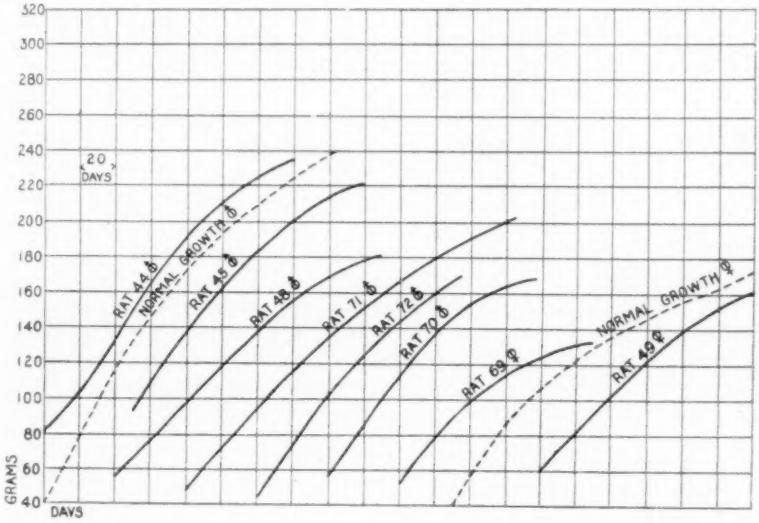


CHART III. Growth on a diet in which coconut press cake was the sole source of protein. The protein content of the press cake was 17.5 (N × 5.7). The diet therefore contained only 13.1 per cent of protein when all the nitrogen in the press cake was calculated as protein nitrogen. Nevertheless the rats grew at an almost normal rate. The composition of the diet was as follows:

	per cent
Coconut press cake	75
Salt mixture	4
Butter fat	21

that coconut press cake contains a sufficient quantity of water-soluble vitamine. There are also indications that it contains some, although an insufficient quantity, of fat-soluble vitamine, since considerable growth was obtained without the addition of butter fat. When the latter was added, the rate of growth became more rapid, as shown in Chart IV.

Preliminary experiments show that coconut press cake is deficient in inorganic constituents, probably calcium, phosphorus, and chlorine.

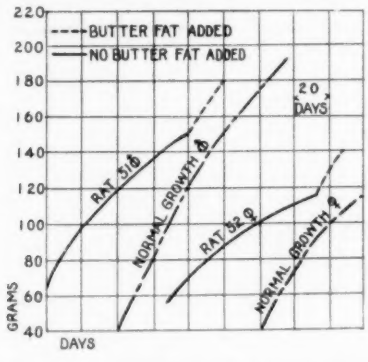


CHART IV. Growth on coconut press cake without the addition of butter fat for a period of 80 days. The growth obtained indicates the presence of some fat-soluble vitamine in coconut press cake. The rate of growth was increased by the addition of butter fat. The composition of the diet during the period when it contained no butter fat was as follows:

	per cent
Coconut press cake	75
Salt mixture	4
Lard	21

During the second period the lard was replaced by butter fat.

CONCLUSIONS.

1. It has been shown that the globulin of the coconut produces normal growth when used as the sole source of protein in an otherwise complete diet.

2. Commercial coconut press cake furnishes the necessary protein for growth at almost a normal rate.

3. Coconut press cake contains sufficient water-soluble vitamine, and experiments indicate that it also contains some fat-soluble vitamine, but the rate of growth is increased by adding butter fat to the diet.

NOTE ON THE DETERMINATION OF BLOOD SUGAR BY THE MODIFIED PICRIC ACID METHOD.

By STANLEY R. BENEDICT.

(From the Department of Chemistry, Cornell University Medical School, New York City.)

(Received for publication, February 26, 1919.)

In a recent number of this *Journal* Rohde and Sweeney[1] have called attention to the fact that certain samples of the picrate-picric acid solution recommended by the present writer for use in the determination of sugar in blood may fail to precipitate properly proteins or chromogenic substances from the blood. In such instances the final results may be many times too high.

We noted this same fact in our laboratory early this fall, but did not connect it, as Rohde and Sweeney have done, with different samples of picric acid. One of our students made up a solution of the picrate-picric acid solution which failed to precipitate blood properly, though other solutions prepared from the same sample of picric acid worked satisfactorily. A study of the question showed that the important point from the practical standpoint is simply one of the final acidity of the solution. For proper precipitation of the blood the solution must have an acidity as high as 0.05 N or 0.04 N, as determined by titration of a portion of the solution with alkali, using phenolphthalein as indicator. The unsatisfactory solutions are usually alkaline to litmus, due either to impurity in the original sample of picric acid, or to addition of a little too much alkali during preparation of the solution. On account of the difficulty of weighing sodium hydroxide with exactness it is preferable to prepare the 500 cc. of 1 per cent solution by dilution of a 10 or 20 per cent solution.

Titration of the final solution is necessary only when a portion fails to precipitate the blood properly. Such a solution may be corrected by addition of a quantity of glacial acetic acid calcu-

[1] Rohde, A., and Sweeney, M., *J. Biol. Chem.*, 1918, xxxvi, 475.

lated to bring the acidity between 0.05 N and 0.04 N. Any excess of acid over that necessary is to be avoided, since even very small amounts of acetic acid in excess will lead to a remarkable decrease in the rate of ultimate color development, both in blood and in pure glucose solutions.

THE PRESENCE OF CALCIUM IN THE RED BLOOD CORPUSCLES OF OX AND MAN.*

By DAVID MURRAY COWIE and HENRIETTA A. CALHOUN.

(From the Department of Pediatrics, University of Michigan, Ann Arbor.)

(Received for publication, September 4, 1918.)

The work of Gryns, Eykman, Overton, Köppe, Hamburger, and of Hedin,[1] goes to show that the blood cells are impermeable to calcium, strontium, barium, and magnesium.

Marriott and Howland[2] state that "corpuscles contain no calcium, and that the fibrin clot contains only a very small and fairly constant amount of calcium." On the other hand, the older chemists, Schmidt[3] in particular, held that the corpuscles contain appreciable amounts of calcium.

Because of this difference of opinion, we attempted to determine whether the red blood corpuscles contain calcium. Methods have been devised by Laws and Cowie[4] and by Lyman.[5] The calcium method for blood using the nephelometer (latest type of Duboscq colorimeter with nephelometer attachment) chosen for the demonstrations was that of Lyman. We make the following slight modifications.

The blood is drawn from the vein in the arm into a 5 cc. pipette attached to a hollow needle by means of a rubber tubing. Before using, the pipette is washed with cold 1 per cent sodium citrate solution made up with 0.9 per cent NaCl solution. This is so successful that, working

* Preliminary report read before the American Pediatric Society, White Sulphur Springs, May, 1917.

[1] Quoted by Hammarsten, O., A text book of physiological chemistry, New York, 5th edition, 1908, 195.

[2] Marriott, W. McK., and Howland, J., *J. Biol. Chem.*, 1917, xxxii, 233.

[3] Schmidt, C., Charakteristik der epidemischen Cholera, Leipsic, 1850; quoted in Mathews, A. P., Physiological Chemistry, New York, 1915, 462.

[4] Laws, C. H., and Cowie, D. M., *Am. J. Dis. Child.*, 1917, xiii, 236.

[5] Lyman, H., *J. Biol. Chem.*, 1917, xxix, 169.

rapidly, three 5 cc. pipettes may be filled without removing the needle from the vein.

After the addition of 1 cc. of the 20 per cent sodium acetate solution, the flasks are cooled on ice until a cloud appears. The flasks are corked with well fitting corks that have been soaked in hydrochloric acid solution and washed with distilled water until the wash water gives no test for acid. All the centrifugalizations are at high speed for 3 minutes. The supernatant fluid is poured off the calcium oxalate at the bottom of the tube with a single rapid motion.

Otherwise, the method follows Lyman's directions, and the calculation is made in mg. of *calcium* (not calcium oxide) per 100 cc. of blood. The ammonium stearate solution keeps well. A solution made in June, 1917, is still clear after standing for a year. All chemicals are tested to be sure that they are calcium-free; for the greater part Kahlbaum salts are used.

Ox Blood.—Ox blood is obtained by collecting it directly from the heart into a clean flask surrounded by a freezing mixture of ice and salt. 5 cc. of this are measured as rapidly as possible into Erlenmeyer flasks containing 15 cc. of trichloroacetic acid. The measuring pipette is rinsed in 1 per cent sodium citrate solution to prevent clotting, and the same pipette is used for all the measurements.

Serum and Corpuscles.—To obtain serum and corpuscles from defibrinated blood, fresh blood is defibrinated at once by whipping with a glass stirring rod. Small pieces of clot are subsequently removed by straining through two layers of surgeon's gauze which has been previously rendered calcium-free by soaking in dilute hydrochloric acid solution, and the acid is removed by washing in calcium-free distilled water until no trace of acid is left. This gives a suspension of corpuscles in serum. The corpuscles and serum are separated by centrifugalization. The serum is pipetted off with a suction pipette.

Unwashed Corpuscles.—To the centrifugal tubes from which the serum has been removed 0.9 per cent sodium chloride solution is added. The tubes are washed once to remove serum that may be adherent to the cells, and then centrifugalized for 10 minutes. The supernatant fluid is pipetted off. Comparative analyses are made on the same batch of corpuscles, 5 cc. samples being used.

Washed Corpuscles.—Washed corpuscles are obtained by washing through three changes of 0.9 per cent salt solution. The isotonic solution prevents the removal of adsorbed calcium but favors the removal of absorbed calcium. It requires an infinite number[6] of washings to remove adsorbed

[6] Bayliss, W. M., Principles of general physiology, London, 2nd edition, 1918, 69.

substances in isotonic equilibrium. 5 cc. samples of washed corpuscles were used for the calcium determinations.

Following the custom used in obtaining solutions containing a certain percentage of red corpuscles in Wassermann technique, we consider the corpuscles in the bottom of the tube after all the fluid has been pipetted off a constant solution. Any variation between different batches is of minor importance, since the object is not to find a method for determining the calcium content of the blood by using the red corpuscle, but to determine whether calcium is present in the corpuscles.

The serum from undefibrinated blood is obtained by allowing the blood to stand 12 to 24 hours until the serum separates. This serum is decanted and centrifugalized to free it from corpuscles. The serum from the bottom of the vessel may contain enough corpuscles to use for the determination of calcium in corpuscles from undefibrinated blood. If there is any hemolysis, the specimen is discarded.

Our results are given in Table I.

TABLE I.

Calcium Content of Whole Blood in Ox and Man, Mg. per 100 Cc.

Whole blood.	No. of determinations.*	Calcium.
		mg.
Ox blood.		
No. 1	11	8.68
" 2	10	7.38
" 3	7	7.40
Average		7.82
Human blood.		
Case 1	15	6.64
" 2	7	8.92
" 3	1	8.12
" 4	1	7.82
" 5	1	9.08
" 6	1	8.82
" 7	1	9.06
" 8	1	8.79
" 9	1	8.82
Average		8.45

* For each determination an average of six readings was taken. The unknown was always set at 32.

From Table I it will be seen that three healthy oxen gave an average of 7.82 mg. of calcium per 100 cc. in twenty-eight

determinations. The mean variation from the average for each animal in no case exceeded 0.5 mg. per 100 cc. Nine healthy men gave an average of 8.45 mg. of calcium per 100 cc. of whole blood in twenty-nine determinations.

The limits of variation of blood calcium in normal individuals are marked, as is shown in the two men, Cases 1 and 2. Lyman[5] found the same variations in normal individuals, which he was unable to explain. Determinations of the number of corpuscles and of the specific gravity of the blood might aid in clearing up this point by giving an idea of the concentration.

TABLE II.

Calcium Content of Normal Serum and of Serum from Defibrinated Blood in Ox and Man. Mg. per 100 Cc.

Serum.	No. of determinations.	Calcium.
		mg.
Ox 1, undefibrinated*	12	8.05
" 1, defibrinated†	7	8.42
" 2, undefibrinated	5	9.13
" 2, defibrinated	3	9.08
Average		8.67
Man (Case 2), undefibrinated	4	12.07
" (" 2), defibrinated	3	12.08
Average		12.07

* Blood allowed to clot and serum pipetted off.

† Fibrin removed at once by defibrination.

From Table II it will be seen that two healthy oxen show an average calcium content of 8.67 mg. of calcium per 100 cc. of blood serum in twenty-seven determinations.

One healthy man gave an average calcium content of 12.07 mg. per 100 cc. of blood serum in seven determinations. It is of interest to recall that this man's whole blood calcium content was also high (8.9 mg.).

It will be seen that defibrination of the blood makes no difference in the calcium content of the serum. This also proves that *if* calcium adheres to the fibrin there is no further increase in the amount adhering after the initial formation of the fibrin threads.

The number of readings on the corpuscles (Table III) would be inadequate if an attempt were being made to determine the amount of calcium actually in the corpuscle itself. But, since the determinations show that the corpuscles constantly contain calcium but in lower concentration than the plasma, we must consider

TABLE III.

Calcium Content of Washed and Unwashed Defibrinated Blood Corpuscles in Ox and Man, Mg. per 100 Cc.

	No. of determinations.	Calcium.
		mg.
Ox 2, unwashed blood corpuscles	8	4.98
" 2, washed " "	4	2.07
Man,* unwashed " "	8	4.26
" washed " "	4	3.47

* Case 2.

TABLE IV.

Calcium Partition or Distribution in Ox Blood and in Human Blood.

Portion of blood taken.	Mg. of Ca in 100 cc.	
	Ox.	Man.
Whole blood	7.82	8.45
Serum from defibrinated and undefibrinated blood	8.67	12.07
Washed corpuscles from defibrinated blood	2.07	3.47
Unwashed corpuscles from undefibrinated blood.	4.98	4.26

that the corpuscle calcium content affects the total amount of calcium in the circulation. To facilitate comparison Table IV is presented.

CONCLUSION.

The red blood corpuscles contain calcium, but in a somewhat smaller concentration than the serum.

NOTE ON THE STABILIZATION OF DILUTE SODIUM HYPOCHLORITE SOLUTIONS (DAKIN'S SOLUTION).

By GLENN E. CULLEN and ROGER S. HUBBARD.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, January 22, 1919.)

The preparation of dilute sodium hypochlorite solutions electrolytically is so economical and convenient that it was desirable to determine a simple method of stabilizing it for use as Dakin's solution.

We have compared the influence of various substances on the rate of decomposition of dilute sodium hypochlorite solutions.

Dakin[1,2] set the upper limit of alkalinity of clinical hypochlorite solutions at the end-point of powdered phenolphthalein and used buffer salts to maintain the alkalinity below this limit. He neutralized with boric acid the excess alkalinity of the filtrate from the sodium carbonate and bleaching powder. The original Dakin's solution, therefore, contained both carbonate and borate buffers. Daufresne[3] modified Dakin's method by substituting sodium bicarbonate for the boric acid. The resulting solution contained only carbonate buffers.

Cullen and Austin,[4] studying the alkalinity of dilute sodium hypochlorite solutions containing carbonates, found that the end-point with powdered phenolphthalein was at a hydrogen ion concentration of about 10^{-10} N (pH 10), and that a reduction of the alkalinity below a pH of 9 made the solution too unstable to be of clinical use. The alkalinity of Dakin's solution, therefore, must be between 100 and 1,000 times that of water. The lower limit of alkalinity may be approximately detected by the use of alcoholic solution of phenolphthalein or *o*-cresolphthalein. The desirability

[1] Dakin, H. D., *Brit. Med. J.*, 1915, ii, 318.

[2] Dakin, H. D., and Dunham, E. K., Handbook of antiseptics, New York, 2nd edition, 1918, 116.

[3] Daufresne, M., *Presse méd.*, 1916, xxiv, 474.

[4] Cullen, G. E., and Austin, J. H., *J. Biol. Chem.*, 1918, xxxiv, 553.

of maintaining the alkalinity within this zone has been further emphasized by the observation that dilute hypochlorite solutions with an alkalinity less than indicated by a pH of 9, as well as those with an alkalinity greater than indicated by a pH of 10, are much more irritating than those with a pH between 9 and 10 (Cullen and Taylor).[5]

Daufresne has stabilized electrolytic sodium hypochlorite solutions by the use of small amounts of NaOH.[6] Although such solutions, unlike those originally described by Dakin, contain no buffer salts, they nevertheless conform to his requirements in concentration and alkalinity, and, therefore, it appears justified to call them Dakin's solutions.

EXPERIMENTAL.

The following substances were tested:

Borates.—Since Dakin used the highly efficient buffer action of borates, it was logical to test the stabilizing influence of borates on electrolytically prepared sodium hypochlorite solutions. Fortunately, the addition of borax ($Na_2B_4O_7.10H_2O$) so adjusted the reaction that there was no color to powdered phenolphthalein, but definite color with alcoholic phenolphthalein or *o*-cresolphthalein. Borax was added in a concentration of from 0.25 to 1.5 per cent.

Carbonates.—Three sets of carbonate and bicarbonate mixtures, having pH values of 10, 9.5, and 9 respectively, were prepared and added to the test solution in concentrations of 0.5, 1, and 1.5 per cent. McCoy's[7] formula was used in calculating the ratio of carbonate to bicarbonate.

$$\frac{NaHCO_3}{Na_2CO_3} = C_H \times \frac{0.8}{6.0 \times 10^{-11}}$$

that is, for pH of 10.0 1 molecule Na_2CO_3 to 1.3 molecules $NaHCO_3$
" " 9.5 1 " " " 4.2 " "
" " 9.0 1 " " " 13.3 " "

[5] Cullen, G. E., and Taylor, H. D., *J. Exp. Med.*, 1918, xxviii, 681.

[6] Personal communication. Daufresne adds NaOH to the solution in the proportion of 0.2 gm. of NaOH to 5 gm. of NaOCl; *i.e.*, if he prepared a 1 per cent NaOCl solution he would add 0.4 gm. per liter. This solution keeps for months. He uses a special cell with platinum and silver electrodes and probably has much less secondary decomposition produced with this cell.

[7] McCoy, H. N., *Am. Chem. J.*, 1903, xxix, 437.

Na_2CO_3 alone, of course, makes the solution too alkaline, and $NaHCO_3$ alone accelerates the rate of decomposition by greatly reducing the alkalinity.

In order to determine the pH of the hypochlorite solutions after the addition of carbonate, 10 cc. samples were decomposed with hydrogen peroxide and titrated with 0.1 N HCl to methyl orange and phenolphthalein. As shown in Table I the pH of the hypochlorite solutions after the addition of 1 per cent carbonate is about 0.2 pH less than calculated for the ratio of sodium carbonate and bicarbonate added.

With impure sodium chloride or with water of high calcium or magnesium content a rather heavy cloud will form in the solution after the addition of carbonate. This will settle out on standing or may be removed by filtration.

TABLE I.

1 Per Cent NaOCl Made from 8 Per Cent NaCl Diluted to 0.5 Per Cent NaOCl with 2 Per Cent Carbonate Solution and Water.

pH of 2 per cent carbonate solution.	Carbonate. Final concentration.	Final pH of hypochlorite solution.
	per cent	
10	0.25	8.9
10	0.5	9.5
10	0.75	9.7
10	1.0	9.8
9.9	0.25	8.7
9.9	0.5	9.2
9.9	0.75	9.6
9.9	1.0	9.7
9.6	0.5	8.8
9.6	1.0	9.4

Sodium Hydroxide.—NaOH was added to the solution in amounts from 0.15 to 0.3 gm. per liter. Over 0.03 per cent makes the solution too alkaline; that is, it gives color with powdered phenolphthalein.

These substances were tested in two ways: First, current was passed through an electrolytic cell, filled with brine, just long enough to produce 0.5 per cent NaOCl. The solution was with-

drawn from the cell, thoroughly mixed, and measured portions were placed in dark brown bottles containing the desired quantity of hydroxide or buffer. Second, the current was passed until the NaOCl was about 0.7 to 0.8 per cent, that is, near the crest of the curve.[8] The solution was then removed and diluted to 0.5 per cent with water. In this case the stabilizing substances were added in concentrated solutions. With this procedure more secondary products are formed (see following paper), the solutions are more unstable, and the test, therefore, more severe.

Several experiments were run under varying conditions. In some the solutions were kept in brown bottles; in others they were exposed to light to accelerate decomposition.

Two representative experiments are given in Tables II and III. Any change in the alkalinity of these solutions during the period of the experiments was within the range of alkalinity indicated by the end-points of alcoholic and powdered phenolphthalein and *o*-cresolphthalein.

TABLE II.

10 Liters of 3 Per Cent NaCl. 110 Volts. 14 to 16 Amperes. Initial Temperature 18°C. 15 Minutes. NaOCl=0.5 Per Cent.

Treatment of solution.		Sodium hypochlorite concentration.	
Substance.		Initial.	After 7 days in brown bottle.
	per cent		
Control		0.5	0.35
Borax	0.5	0.5	0.48
"	0.25	0.5	0.465
NaOH	0.3	0.5	0.49
"	0.2	0.5	0.46
"	0.015	0.5	0.42
pH 10 carbonate	1.0	0.5	0.49
" 9.5 "	1.0	0.5	0.45
" 9 "	1.0	0.5	0.41

[8] Cullen, G. E., and Hubbard, R. S., *J. Biol. Chem.*, 1919, xxxvii, 519, Fig. 2, Curve C.

It is evident from our experiments that 0.2 to 0.3 gm. per liter of sodium hydroxide, borax in concentration of 0.25 per cent up, carbonate mixture of pH 10 from 0.5 per cent up, and carbonate mixture of pH 9.5 from 1.0 per cent up, maintain dilute NaOCl solution within the requirements of Dakin's solution for at least a week. The pH 9 carbonate mixture allows somewhat more rapid decomposition. If sodium hydroxide is used, care must be taken that the amount is accurately measured, or the alkalinity may be made dangerously high. The use of borax combines a maximum of convenience and safety.

TABLE III.

10 Liters of 3 Per Cent NaCl. 110 Volts. 20 Minutes. Initial Temperature 7°C. 0.75 Per Cent NaOCl Diluted to 0.50 Per Cent.

Treatment of solution.		Sodium hypochlorite concentration.		
Substance.		Initial.	After 7 days in colorless bottle.	After 26 days in brown bottle.
	per cent			
Control		0.5	0.29	0.09
Borax	1.5	0.5	0.48	0.50
"	1.0	0.5	0.48	0.45
"	0.5	0.5	0.47	0.41
NaO H	0.03	0.5	0.49	0.48
"	0.02	0.5	0.48	
"	0.015	0.5	0.42	0.23
pH 10 carbonate	1.5	0.5		0.49
" 10 "	1.0	0.5	0.49	0.48
" 10 "	0.5	0.5		0.47
" 9.5 "	1.5	0.5		0.48
" 9.5 "	1.0	0.5	0.45	0.47
	0.5	0.5		0.43

DISCUSSION.

The solvent action of the sodium hypochlorite solution is an essential factor in its germicidal efficiency. The necrotic tissue, pus, etc., that protect the bacteria from many antiseptics are dissolved

away by the hypochlorite solution. Fiessinger and his coworkers have attributed this solvent action mainly to the alkalinity of the solution, but Taylor and Austin[9] have shown that within the zone pH 9 to pH 10 the solvent action is primarily due to the hypochlorite. However, when no alkali was added to the hypochlorite solution and the alkalinity was due entirely to the dissociation of sodium hypochlorite, solvent action ceased when the hypochlorite concentration was reduced below 0.2 per cent.

Solution of the protein material probably takes place in two steps: first, the chlorination of the protein, and second, the formation of soluble sodium salts of the chlorinated proteins. The chlorination of the protein, as indicated by the rate of the decomposition of the hypochlorite solution, is rapid when large amounts of pus and necrotic tissue are present, and the initial 0.5 per cent concentration is maintained in the wound for only a few minutes. When the alkalinity is maintained by buffer salts, the solvent action occurs not only during this period but presumably until all the products capable of reacting are changed to the soluble sodium salts. When the alkalinity is due to small quantities of free alkali, as sodium hydroxide, the concentration of alkali should be sufficient to maintain the alkaline reaction long enough for efficient solvent action. On the other hand, the concentration must not be so great that free alkali remaining unneutralized in the wound is sufficient to cause irritation. The concentration used by Daufresne —0.2 to 0.3 gm. per liter—seems to satisfy these conditions. Experience at the War Demonstration Hospital of The Rockefeller Institute has not yielded clinical evidence to warrant choosing between the two types of hypochlorite solutions provided they conform to Dakin's conditions of hypochlorite concentration and alkalinity.

SUMMARY.

There are two methods of applying Dakin's principle of low alkalinity: first, by maintaining the alkalinity at a pH of 9 to 10 by means of buffer salts, such as carbonate or borates; second, by maintaining a similar alkalinity by means of *small* amounts of alkali. Both methods are efficient.

[9] Taylor, H. D., and Austin, J. H., *J. Exp., Med.*, 1918, xxvii, 155.

0.5 per cent sodium hypochlorite, prepared by the electrolysis of sodium chloride, may be conveniently stabilized for use as Dakin's solution by the addition of 0.5 per cent borax, of 0.5 to 1.0 per cent of carbonate mixtures of pH 10 to 9.5, or by the addition of 0.2 gm. of sodium hydroxide per liter (Daufresne).

NOTE ON THE ELECTROLYTIC PREPARATION OF DILUTE SODIUM HYPOCHLORITE SOLUTIONS (DAKIN'S SOLUTION).

By GLENN E. CULLEN and ROGER S. HUBBARD.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, January 22, 1919.)

Although the electrolysis of brine is an old process, information in regard to it is scattered and hidden in the records and patent literature of industrial concerns. Dakin and Carlisle[1] have pointed out its convenience and economy in preparing dilute sodium hypochlorite solutions for disinfection purposes and have designed a simple cell. In instructing army surgeons in the various methods of preparing Dakin's solution, it was desirable for us to determine for ourselves the factors that were of practical importance in its electrolytic preparation. These results are presented for the convenience of other workers.

EXPERIMENTAL.

Choice of Cell.—It seemed desirable to use a cell that could be connected with ordinary 110 volt current, that did not require unusually heavy wiring or power, that was light and strong enough to be transportable with other military hospital equipment, and that was inexpensive. The cell described by Dakin and Carlisle answers these requirements, but we were spared the labor of making this cell by modifying cells already on the market to our purpose. These cells are entirely similar to that described by Dakin and Carlisle, except that the electrode area is smaller in proportion to the volume of solution. The twenty-three intermediate elec-

[1] Dakin, H. D., and Carlisle, H. G., *J. Roy. Army Med. Corps*, 1916, xxvi, 209. See also Dakin, H. D., and Dunham, E. K., Handbook of antiseptics, New York, 2nd edition, 1918, 116.

trodes contained 30 square inches of Acheson graphite. The cell[2] used in these experiments held 10 liters of brine and required between 20 and 35 amperes.

The results are presented as curves, with the omission of the tables from which they were derived.

Influence of Temperature.—In order to determine the effect of the initial temperature of the solution, the current through the cell was maintained constant at 20 amperes by an external resistance.[3] This

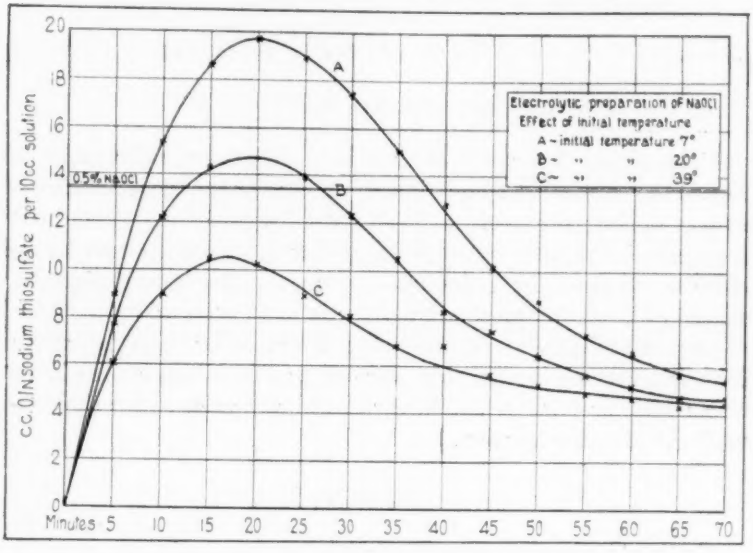


FIG. 1.

eliminates the influence of current fluctuations due to change in internal resistance. Fig.1 shows the results with initial temperatures of 7, 20, and 39°.

Influence of Salt Concentration.—Increase in salt concentration will, of course, lessen the internal resistance of the cell and consequently increase the production of sodium hypochlorite per unit

[2] This cell was furnished by courtesy of the Electro Chemical Company of Dayton, Ohio.

[3] The resistance units used to control the lights in theaters are convenient for this work.

of time. The curves in Fig. 2 show the production of sodium hypochlorite with different salt concentrations under actual operating conditions, with no external resistance.

The 3 per cent solution approximates sea water, and 6 per cent is the strength recommended by the makers. More concentrated solutions may, of course, be used and with them higher concentration of sodium hypochlorite and increased current efficiency may be obtained.

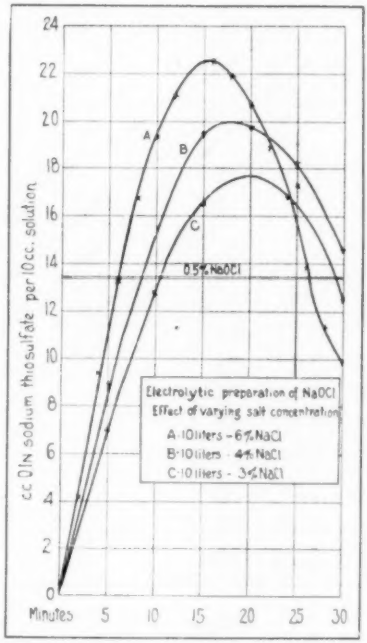
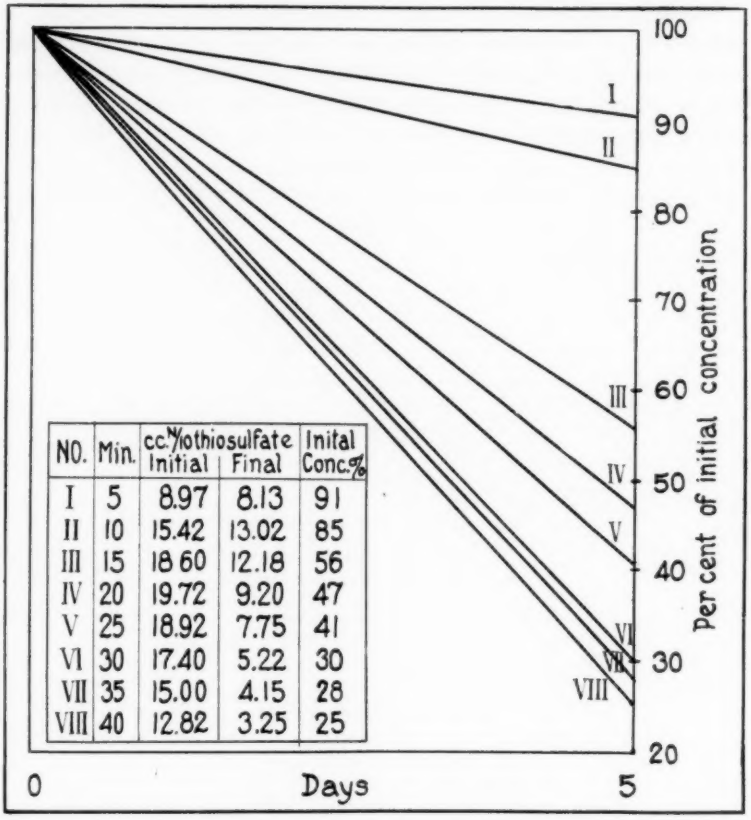


FIG. 2.

Rate of Decomposition of Electrolytic Sodium Hypochlorite.—In the experiments shown above samples of solution were removed from the cell at each of the points shown, titrated, and a portion was set aside for determination of stability. The solution became increasingly unstable with increase in hypochlorite concentration. The results obtained from Curve A, Fig. 1, run at initial temperature of 7°, are plotted on Fig. 3. This increased rate of decomposition is due to increase in secondary products rather than

to the temperature at time of sampling, for in one series in which the samples were all cooled to the same temperature the results were essentially similar.



NO.	Min.	cc. Thiosulfate Initial	Final	Inital Conc.%
I	5	8.97	8.13	91
II	10	15.42	13.02	85
III	15	18 60	12.18	56
IV	20	19.72	9.20	47
V	25	18.92	7.75	41
VI	30	17.40	5.22	30
VII	35	15.00	4.15	28
VIII	40	12.82	3.25	25

FIG. 3.

DISCUSSION.

It would seem desirable to construct from experimental runs, curves similar to those of Figs. 1 and 2, for each cell. Then, from the initial temperature of the solutions, the time required for a solution of given concentration may easily be determined. Care should be taken that the cell is not operated beyond the peak of the production curve. Ordinarily 3 per cent NaCl (or sea water) is

satisfactory, but if necessary to operate with relatively warm solution the salt concentrations should be increased.

Since the solution, as it comes from the cell, decomposes quickly, it must be stabilized for use as Dakin's solution. As determined in the preceding paper, this can best be accomplished by adding either 0.5 per cent borax, or 0.5 to 1.0 per cent of a mixture of carbonate and bicarbonate of pH 10 to 9.5, or 0.02 per cent sodium hydroxide.

This solution must give no color with powdered phenolphthalein but should give a definite red flash with alcoholic phenolphthalein solution. It should be protected from light and should be titrated frequently.

THE CHEMICAL INVESTIGATION OF SPOILED MEAT.

By K. GEORGE FALK, EMIL J. BAUMANN, and GRACE McGUIRE.

(From the Harriman Research Laboratory, The Roosevelt Hospital, New York, in Cooperation with the Division of Food and Nutrition, Medical Department, U. S. Army.)

(Received for publication, February 21, 1919.)

The investigations here described are part of a general study of the problems arising in connection with food poisoning. While the cause of such poisonings is not definitely known, it is often associated with bacteria in spoiling food. The symptoms of poisoning are probably due either to toxins produced in the course of the metabolism of the organisms, or to an infection of the intestinal tract. Whatever may be the exact relation of bacteria found in spoiling food to the illness caused by its ingestion, it would be desirable to develop a chemical test by means of which the early stages of spoilage could be detected. This was one of the objects of the present study. Experimentation was limited to spoiled meat, this being the food most frequently suspected of causing poisoning. Also, the experiments were conducted for the most part on samples inoculated with organisms isolated from meat thought to be responsible for actual cases of poisoning. The general method employed was to study the chemical changes taking place in certain components of the meat as a result of the action of the selected organism. The components thus investigated in the meat under observation were: (1) total nitrogen, (2) non-protein nitrogen, (3) ammonia nitrogen, (4) total creatinine nitrogen (creatine plus creatinine), (5) purine nitrogen. Previous investigators studied the changes in meat in cold storage at temperatures below and above freezing.[1] As a rule, the chemical constituents determined differed from those determined in this investigation.

[1] Richardson, W. D., and Scherubel, E. F., *J. Am. Chem. Soc.*, 1908, xxx, 1515; *J. Ind. and Eng. Chem.*, 1909, i, 95. Emmett, A. D., and Grindley, H. S., *ibid.*, 413. Wright, A. M., *J. Soc. Chem. Ind.*, 1912, xxxi, 965. Hoagland, R., McBryde, C. N., and Powick, W. C., *U. S. Dept. Agric., Bull. 433*, 1917.

Analytical Methods.

A rather full description of the methods used in the estimations will be given in the following paragraphs, both on account of the importance of these methods to the experiments here reported and in view of the fact that they may have an interest of their own.

In outline the procedure adopted was as follows: On a small portion of each lot of meat in which spoilage was investigated, the total nitrogen and ammonia nitrogen were first determined. The protein substances were then coagulated in a large sample of the meat and the non-protein substances separated from the coagulum by washing with water. Upon the water solution of the non-protein substances thus separated, determinations were made of nitrogen, purine nitrogen, total creatinine nitrogen, and ammonia nitrogen. It may be noted at this point that the difficulties encountered in applying methods already worked out arose as a consequence of the chemical and physical nature of the decomposed meat. This rendered necessary certain minor changes in the analytical methods originally designed for the fresh product.

Total Nitrogen.—Total nitrogen was determined directly on a 1 gm. sample of the meat by the Kjeldahl-Arnold-Gunning method.

Ammonia. (*a*) *In meat.*—1 to 3 gm. of uniformly hashed meat were weighed, placed in a large test-tube (8″ × 1″), treated with 5 to 15 cc. of water, enough C.P. sodium chloride to form a saturated solution, and 1.2 cc. of 10 per cent sodium hydroxide solution, and aerated for 2 to 3 hours into 10 cc. of 0.1 to 0.01 N acid, the amount of acid depending upon the amount of ammonia present. The excess acid was titrated with standard alkali, using a mixture of methyl red and methylene blue as indicator.[2]

(*b*) *In Filtrates containing Non-Protein Nitrogen.*—25 to 40 cc. of the filtrate were treated with sufficient sodium chloride to form a saturated solution, 1.2 to 1.5 cc. of 10 per cent sodium hydroxide solution were added, and the mixture was aerated as in meat.

Separation of Protein from Non-Protein Constituents.—This proved to be the most difficult part of the investigation. As

[2] The use of this indicator mixture was suggested by Dr. E. M. Frankel who stated that it was devised by Professor Alonzo E. Taylor and Major Caspar W. Miller. It gives an exceedingly sharp color change with 0.01N reagents.

noted above a number of methods previously worked out for fresh meat were tried and while suitable for that, proved unsuccessful when applied to decomposed meat. The method which yielded the most satisfactory results and which was finally adopted consisted in the coagulation of the proteins by means of heat in the presence of alumina cream. The only difficulties encountered in the use of this method were the loss of ammonia during the process of heating and the difficulty of washing the large mass of coagulum and alumina cream free of the non-protein substances. The first of these was overcome by testing a sample of the meat for ammonia before coagulating the protein, while thorough washing of the coagulum according to the technique given below served to avoid the second. The sample of meat (125 gm.) was first finely hashed and added to 400 cc. of boiling water in an aluminium pan with a lip. The meat was broken up by boiling from 1 to 4 minutes, and when fresh meat was used, about 100 cc. of alumina cream were then added. (The alumina cream was prepared by boiling in an open pail a solution of filtered 8 per cent aluminium acetate diluted with about six times its volume of water for 8 to 12 hours, the water being replaced as evaporation occurred. The suspension was then filtered through canvas bags. This method, suggested by Dr. Frankel, is satisfactory and applicable on a large scale.) In badly decomposed meat, 250 cc. of alumina cream were necessary, while meat in intermediate stages of decomposition was treated with an amount of alumina cream between these two limits. After the addition of the alumina cream, the mixture was again brought to the boiling point, stirred, heating continued for another half minute, and the mass then filtered through a large filter paper (15 inches). If the coagulation has been properly carried out, a clear, faintly yellow filtrate is obtained. The mixture was allowed to drain completely and the contents of the filter paper were very carefully returned to the aluminium pan by means of a porcelain spoon. The meat, which had clumped together during the coagulation, was broken apart with the spoon into small bits. About 300 cc. of water were added to the coagulum in the pan, the mixture was brought to a boil, and filtered as before. This washing and scooping out was repeated three times, making four washings in all. Finally, the filter paper and contents were washed once with about 100 cc. of hot water and the filtrate and washings

made up to 3,000 cc. As the meat decomposed, it became more difficult to remove protein hydrolysis products and it was necessary to add a little alumina cream with each boiling. The meat also became very rubbery. If a perfectly clear filtrate was not obtained, the filtrate had to be brought to a boil and more alumina cream used.

In preliminary experiments, the coagulation of the protein was also accomplished by colloidal ferric hydroxide and magnesium sulfate. This method was used upon water extracts of the meat; the procedure was carried out in two ways. In the first, the hydroxide and the magnesium sulfate acted upon an extract from which the particles of meat were not removed. In the second case, the reagents were allowed to act upon the extract after straining out the meat particles. The procedure finally adopted, however, was the one described in detail above, since it was found that by this means the best separation of protein from non-protein material was accomplished.

Creatine plus Creatinine.—Creatine plus creatinine[3,4] was determined as follows, on a portion of the non-protein nitrogen filtrate equivalent to 1 to 2 gm. of meat: 5 cc. of normal hydrochloric acid solution were added to the portion of the filtrate and the solution was evaporated to about 2 cc. in a 50 cc. conical flask on an electric stove. The flask was then covered with a watch-glass and heated on low heat on the electric plate for 4 to 5 hours to convert the creatine into creatinine at a temperature just below boiling. When cool, the acid was neutralized by a 10 per cent solution of sodium hydroxide, with one drop of a 0.1 per cent methyl red solution as indicator. Immediately upon neutralization, 15 cc. of a pure saturated picric acid solution and 3 cc. of 10 per cent sodium hydroxide solution were added. The solution was then allowed to stand 10 minutes to permit the color to develop, diluted, and compared in a colorimeter with a standard creatinine zinc chloride solution.

Purine Nitrogen.—Purine nitrogen[5] was determined in the following manner: About two-thirds to three-fourths of the entire

[3] Janney, N. W., and Blatherwick, N. R., *J. Biol. Chem.*, 1915, xxi, 567.

[4] Greenwald, I., and McGuire, G., *J. Biol. Chem.*, 1918, xxxiv, 107.

[5] Modification of the method of Krüger and Schmid, *Z. physiol. Chem.*, 1905, xlv, 1.

filtrate (usually 2,000 cc.) was first evaporated to 50 cc. under diminished pressure (temperature 40–45°). If necessary, caprylic alcohol was added from time to time to prevent frothing during this evaporation. The concentrated solution was carefully washed into a 250 cc. beaker, the final volume being made up to 100 to 150 cc., and the purines precipitated as the silver magnesium compounds. The precipitation was accomplished by first making the solution distinctly alkaline with ammonium hydroxide. 10 cc. of an ammoniacal silver nitrate solution were then added, this solution being prepared by dissolving 26 gm. of silver nitrate in a liter of water and adding ammonium hydroxide until the precipitated silver hydroxide redissolved. 10 cc. of a 6 per cent disodium phosphate solution were added at this point, and finally 5 cc. of an ammoniacal magnesia mixture solution (100 gm. of magnesium chloride, 200 gm. of ammonium chloride, in 1 liter of water with the addition of ammonium hydroxide until an excess of ammonia was present). Thorough stirring accompanied the addition of each reagent. The solution was allowed to stand 2 hours, filtered on a fluted paper, the precipitate washed four times with a little cold distilled water, and then washed back into the beaker in which the precipitation had been made. The precipitate, consisting of silver-magnesium purine compounds, was then decomposed by adding 10 to 15 cc. of 10 per cent hydrochloric acid solution and allowing to stand in the dark over night. The purine hydrochlorides resulting were filtered into a liter round-bottomed flask, with several bits of cracked porcelain, made slightly alkaline with sodium hydroxide, then distinctly acid with acetic acid and 10 to 15 cc. of 10 per cent acetic acid solution added in excess. The solution was then brought to a boil, and 10 cc. of a 40 per cent sodium bisulfite solution and 10 cc. of a 10 per cent copper sulfate solution successively were added. A white precipitate formed which became brown on boiling. The boiling was continued 3 to 5 minutes. The copper purine compounds were filtered while very hot on a fluted paper and washed thoroughly with hot water. The precipitate was then washed into a Kjeldahl flask and the nitrogen determined by the usual Kjeldahl procedure. It was necessary to add only 15 cc. of sulfuric acid; no potassium sulfate was needed as digestion proceeded rapidly. This method, providing two precipitations, the first in alkaline and the second in

acid solution, gives pure compounds upon which to determine the nitrogen. Experiments were made to determine whether, employing the above procedure, added purines could be recovered quantitatively when allowed to soak up in meat, and it was found that the method gave correct results when purines were thus added.

Total Nitrogen of Filtrate.—This was determined on a 50 cc. portion of the filtrate by the Kjeldahl-Arnold-Gunning method.

Experiments on Meat and Meat Extracts.

The preliminary experiments made on meat, using the methods described, gave the results shown in Table I. In these preliminary experiments, lean meat was hashed and allowed to decompose spontaneously. Each day, after proper mixing, a sample was re-

TABLE I.

Analyses of Fresh and Decomposing Meat.

Date.	Nitrogen percentages of meat.					
	Total N.	NH_3-N in meat.	Non-protein N.	NH_3-N in filtrate.	Creatine plus creatinine N.	Purine N.
Series A. Temperature 2-5°C.						
1917						
Dec. 4			0.36	0.015	0.12	
" 5			0.36	0.017	0.12	
" 6			0.30	0.016	0.10	
			0.34	0.015	0.12	
" 8			0.35	0.020	0.12	
" 10			0.31	0.025	0.12	
" 13			0.29	0.055	0.12	
Series B. Temperature 15°C.						
1918						
Feb. 11	3.66	0.012	0.43	0.009	0.12	0.027
" 13		0.025	0.42	0.030	0.11	0.028
" 15		0.043	0.41	0.049	0.13	0.030
" 16		0.052	0.40	0.059	0.13	0.028
" 17		0.064	0.42	0.070	0.13	0.029
" 18		0.077	0.48	0.081	0.14	0.030
" 19		0.098	0.44	0.094	0.14	0.029
" 20		0.127	0.42	0.11	0.14	0.025
" 21	3.80	0.190	0.44	0.13	0.13	0.020

moved from the mass for analysis. The results given in the table are expressed as percentages of the total weight of the meat. Two series of experiments were conducted, Series A at a comparatively low temperature and Series B at a higher temperature.

Owing to the extreme difficulty, if not impossibility, of working with sterile meat, it was decided at this stage of the investigation to employ meat extracts for all further work. This procedure was thought to be justified in view of the fact that the extracts, as prepared in these studies, contained all the substances present in the meat except the insoluble proteins and the lipins. Extracts were accordingly prepared from lean chilled beef, secured 24 to 48 hours after the animal had been slaughtered. The sample of beef was hashed and steeped with an equal volume of water over night. The extract was squeezed through cheese-cloth the following day and, in order to render it approximately sterile, hydrochloric acid was added until the hydrogen ion concentration was between 10^{-3} and 10^{-4} N. The acidified liquid was then allowed to stand for 1 or 2 hours. Bacterial cultures on agar plates showed that such a medium was fairly effective for killing off most of the microorganisms, the counts varying from 5 to 20 per cc. Before inoculating with test organisms, the acid extract was made alkaline with sodium hydroxide. A voluminous precipitate of acid protein was produced by the hydroxide. Addition of alkali was continued until the hydrogen ion concentration was between $10^{-6.5}$ and 10^{-7} N. The extract was then diluted with sterile water to bring the concentration of sodium chloride to about 0.6 to 0.7 per cent.

This extract was transferred to flasks, with the customary aseptic precautions, approximately the same amount being introduced into each flask. The contents of the flasks were inoculated with 10 cc. each of a broth culture having a rich growth of the test organism. At intervals, usually once a day, a complete analysis was made on the contents of each flask by the methods already described. At the end of the experiments, a drop of the extract was in every case found to be teeming with the organism studied. No evidence of contamination was obtained. Presumably, the organism used for inoculation was numerically so much greater than the few in the extract before inoculation that the latter were killed off. For all practical purposes, therefore, the effects noted in these studies were those of a pure culture. In order to follow

the changes produced in the meat extracts, cultures were kept at room temperature (15–25°), although the optimum temperature for the growth of the organisms studied is 38°.

TABLE II.

Action of Bacillus proteus on Meat Extract.

Experiment A.

	Gm. per liter.							
	Mar. 26	Mar. 28	Mar. 29	Apr. 1	Apr. 2	Apr. 3	Apr. 4	Apr. 5
Total N		1.06	1.04					
Ammonia N	0.024	0.027	0.015	0.030	0.031	0.040	0.046	0.056
Non-protein N	0.499	0.487	0.469	0.478	0.444	0.509	0.485	0.490
" " minus NH_3-N	0.475	0.460	0.454	0.448	0.413	0.469	0.439	0.434
K_1 plus K_2N	0.145	0.139	0.142	0.139	0.136	0.131	0.131	0.124
Purine N	0.034	0.038	0.033	0.036	0.036	0.029	0.028	0.022
Residual non-protein N	0.296	0.283	0.279	0.273	0.241	0.309	0.280	0.288

TABLE III.

Action of Bacillus proteus on Meat Extract.

Experiment B.

	Gm. per liter.						
	Mar. 26	Mar. 28	Mar. 29	Apr. 1	Apr. 2	Apr. 4	Apr. 5
Total N		1.06	1.06				
Ammonia N	0.026	0.025	0.017		0.031	0.044	0.051
Non-protein N	0.513	0.501	0.481		0.476	0.497	0.488
" " minus NH_3-N	0.487	0.476	0.464	0.463	0.445	0.453	0.437
K_1 plus K_2N	0.129	0.142	0.139	0.139	0.139	0.131	0.124
Purine N	0.035	0.037	0.036	0.035	0.037	0.028	0.024
Residual non-protein N	0.323	0.297	0.289	0.289	0.269	0.294	0.289

In the first series of experiments with *Bacillus proteus*, a different procedure from the one just described was employed which, however, did not prove entirely satisfactory and was not used in any other experiment. In this series the extraction was made with a 3 per cent salt solution to remove a higher percentage of the globulin in the meat, sterilized by adding about 8 per cent of ether, shaken,

and allowed to stand 24 hours. The ether was then removed by aspiration with sterile air and the extract diluted with enough sterile water to bring the sodium chloride concentration to about 0.9 to 1.0 per cent. The objections to this method of sterilization were

TABLE IV.

Action of Bacillus coli communis on Meat Extract.

	Gm. per liter.								
	Apr. 10	Apr. 11	Apr. 12	Apr. 13	Apr. 15	Apr. 16	Apr. 17	Apr. 18	Apr. 19
Total N	4.82								
Ammonia N	0.086	0.096	0.099	0.100	0.193	0.386	0.756	1.16	2.08
Non-protein N	1.43	1.37	1.44	1.68	1.80	2.13		2.77	3.48
" " minus NH_3-N	1.34	1.27	1.34	1.58	1.61	1.75		1.61	1.40
K_1 plus K_2N	0.554	0.555	0.523	0.506	0.588	0.481	0.321	0.303	0.085
Purine N	0.109	0.123	0.122	0.147	0.120	0.054	0.008	0.001	0.0002
Residual non-protein N	0.677	0.592	0.695	0.927	0.902	1.21		1.31	1.31

TABLE V.

Action of Bacillus paratyphosus β on Meat Extract.

Strain 180.

	Gm. per liter.									
	Apr. 20 7 p.m.	Apr. 22 10a.m.	Apr. 23 6 p.m.	Apr. 25 10a.m.	Apr. 26 10a.m.	Apr. 27 10a.m.	Apr. 28 10a.m.	Apr. 29 3 p.m.	May 1 3 p.m.	May 7 3 p.m.
Total N	4.75									
Ammonia N	0.083	0.090	0.112	0.133	0.176		0.361	0.706	0.482	1.32
Non-protein N	1.64	1.66	1.17	1.47	1.71		2.29	2.37	2.32	2.88
" " minus NH_3-N	1.56	1.57	1.06	1.34	1.53	1.71	1.93	1.66	1.84	1.56
K_1 plus K_2N	0.521	0.514	0.494	0.496	0.540	0.540	0.560	0.554	0.446	0.427
Purine N	0.118	0.135	0.121	0.102	0.103	0.111	0.100		0.103	0.049
Residual non-protein N	0.921	0.921	0.445	0.742	0.887	1.06	1.27		1.29	1.08

the difficulty of removing the ether and the fact that dilution of the extract to reduce the salt concentration to 0.9 to 1.0 per cent yielded a very dilute broth. Although *Bacillus proteus* is a vigorous organism, this broth did not furnish a suitable substratum for its growth.

Organisms Studied.—Bacillus proteus, Bacillus coli communis, Bacillus paratyphosus β (two strains which had caused meat poi-

TABLE VI.

Action of Bacillus paratyphosus β on Meat Extract. Strain 222.

	Gm. per liter.									
	Apr. 20 1 p.m.	Apr. 22 10 a.m.	Apr. 23 6 p.m.	Apr. 25 10 a.m.	Apr. 26 10 a.m.	Apr. 27 10 a.m.	Apr. 28 10 a.m.	Apr. 29 3 p.m.	May 1 3 p.m.	May 7 3 p.m.
Total N	4.81									
Ammonia N	0.083	0.094	0.116	0.142	0.192		0.401	0.434	0.482	1.64
Non-protein N	1.64	1.42	1.31	1.50	1.78		2.43	2.50	2.31	3.13
" " minus NH_3-N	1.56	1.33	1.19	1.45	1.59	1.73	2.03	1.87	1.83	1.49
K_1 plus K_2N	0.520	0.540	0.513	0.520	0.534	0.540	0.554	0.560	0.560	0.514
Purine N	0.118	0.132	0.123	0.093	0.098	0.097	0.104	0.099	0.102	0.080
Residual non-protein N	0.922	0.658	0.554	0.837	0.958	1.09	1.37	1.21	1.17	0.894

TABLE VII.

Action of Bacillus enteritidis (Gaertner Bacillus) on Meat Extract. Strain 25.

	Gm. per liter.									
	Series 1.					Series 2.				
	June 12	June 15	June 16	June 17	June 18	June 29	June 30	July 1	July 2	July 3
Total N	6.30				6.30					
Ammonia N		0.059	0.590	0.829	1.50		0.070	0.138	0.351	0.533
Non-protein N		1.86	3.92	4.69	6.08		0.908	1.02	1.26	1.36
" " minus NH_3-N	1.76	1.80	3.33	3.86	4.58	0.855	0.838	0.881	0.907	0.829
K_1 plus K_2N	0.704	0.670	0.192	0.384	0.253	0.308	0.312	0.261	0.174	0.134
Purine N	0.133	0.155	0.115	0.110	0.045	0.057	0.063	0.058	0.047	0.041
Residual non-protein N	0.923	0.975	3.02	3.37	4.28	0.490	0.463	0.562	0.686	0.654

soning), *Bacillus enteritidis* (Gaertner bacillus, two strains), *Bacillus subtilis*, and *Streptococcus brevis* were the types used in the experiments. The sources of these organisms will be given elsewhere.[6]

[6] Greenwald, I., unpublished data.

TABLE VIII.

Action of Bacillus enteritidis (Gaertner Bacillus) on Meat Extract. Strain 132.

	Gm. per liter.									
	Series 1.					Series 2.				
	June 14	June 15	June 16	June 17	June 18	June 29	June 30	July 1	July 2	July 3
Total N	6.15				6.15					
Ammonia N		0.046	0.604	0.819	1.50		0.058	0.116	0.292	0.481
Non-protein N		1.88	3.57	4.59	6.20		0.940	1.03		1.36
" " minus NH_3-N	1.76	1.83	2.97	3.77	4.70	0.855	0.882	0.917		0.874
K_1 plus K^2N	0.704	0.673	0.105	0.300	0.205	0.308	0.306	0.253	0.174	0.162
Purine N	0.133	0.141	0.035	0.057	0.005	0.057	0.061	0.066	0.056	0.030
Residual non-protein N	0.923	1.02	2.83	3.41	4.49	0.490	0.515	0.598		0.682

TABLE IX.

Action of Bacillus subtilis on Meat Extract.

	Gm. per liter						
	July 15	July 16	July 17	July 18	July 19	July 20	July 21
Total N	4.58						
Ammonia N	0.054	0.096	0.738	1.14	1.36	1.75	2.02
Non-protein N	1.16	1.33	2.49	2.90	2.97	3.33	3.68
" " minus NH_3-N	1.11	1.23	1.75	1.76	1.61	1.58	1.66
K_1 plus K_2N	0.394	0.151	0.408	0.196	0.164	0.078	0.048
Purine N	0.064	0.064	0.074	0.050	0.040	0.016	0.033
Residual non-protein N	0.652	1.02	1.27	1.51	1.41	1.49	1.58

TABLE X.

Action of Streptococcus brevis on Meat Extract.

	Gm. per liter.						
	July 15	July 17	July 18	July 19	July 20	July 21	July 22
Total N	4.56						4.56
Ammonia N	0.054	0.32	0.90	1.29	1.59	1.75	2.16
Non-protein N	1.16	1.98	2.60	2.92	3.40	3.42	3.80
" " minus NH_3-N	1.11	1.66	1.70	1.63	1.81	1.67	1.64
K_1 plus K_2N	0.394	0.368	0.225	0.218	0.130	0.122	0.152
Purine N	0.064	0.072	0.068	0.060	0.004	0.035	0.042
Residual non-protein N	0.652	1.22	1.40	1.35	1.68	1.51	1.46

Most of the organisms were the colon-typhoid group, which are the organisms chiefly concerned in food poisoning. For comparison, it was desired to determine whether organisms belonging to a totally different group produced changes in the substratum similar to those produced by the colon-typhoid group. For this purpose *Streptococcus brevis* and the spore-forming *Bacillus subtilis* were used. It should be noted in the case of *Bacillus enteritidis*, that the organism grew very rapidly so that the early stages of decomposition were not obtained. The experiments were, therefore, repeated to determine the intermediate changes. These results are given in Table VII under Series 1 and 2 respectively.

The results of the experiments on meat extracts are presented in Tables II to X. The nitrogenous constituents are the most important for determining the nature of the changes occurring in the spoiling meat; Figs. 1 to 9 show the nitrogen content of the various nitrogen-bearing fractions of the extract as percentages of the total nitrogen. The times of action plotted along the abscissæ are not plotted according to scale in some of the figures. For the purposes in view, the results are plotted in equal increments although some of the time intervals were different.

DISCUSSION.

The results presented in the tables and the figures show some marked differences as well as similarities by the actions of the different strains. The creatine-creatinine values change only slightly with *Bacillus proteus* and *Bacillus paratyphosus β*, but decrease markedly with *Bacillus coli communis*, *Bacillus enteritidis*, *Bacillus subtilis*, and *Streptococcus brevis*. The purine values decrease rapidly with *Bacillus coli communis* and *Bacillus enteritidis*. With *Bacillus proteus* and *Bacillus paratyphosus β* the purine nitrogen showed a more or less irregular (accidental) variation for the first action followed by a small decrease. *Bacillus subtilis* and *Streptococcus brevis* gave a distinct final increase in the purine indicating a synthesis. The ammonia increased in every case. The non-protein nitrogen and residual non-protein nitrogen values represent the summation of the changes of a number of substances.

The results show that bacteria exert certain selective actions on definite substances or groups of substances. The results are

not as yet quantitative enough for careful comparisons, but indicate that more careful determinations in which the conditions are controlled more accurately and with synthetic media, wholly or in part, would give a method for distinguishing various strains of bacteria.

The increase in the ammonia content, which is the one common factor in all the actions, may be of value in determining when the meat becomes unpalatable or unsuitable for use by estimating the amount of this constituent present. This question is taken up in the paper which follows.

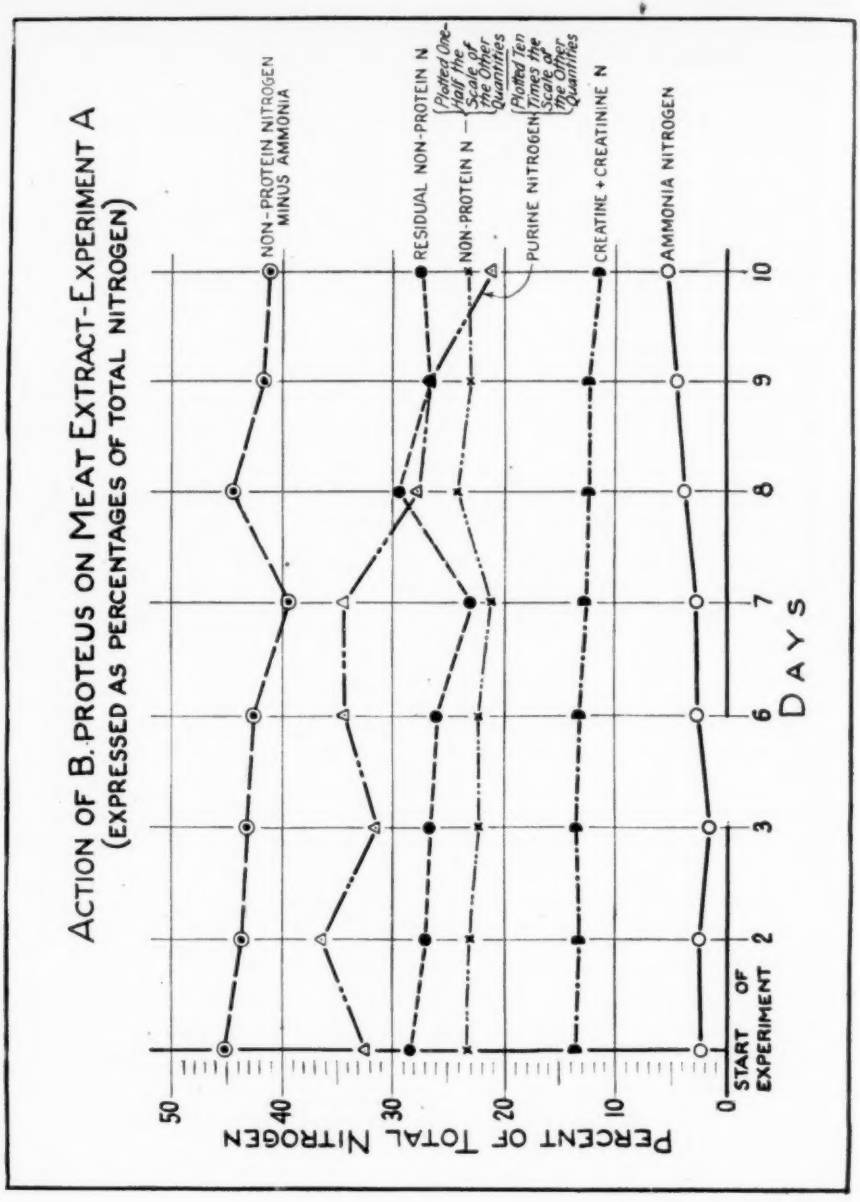

ACTION OF B. PROTEUS ON MEAT EXTRACT-EXPERIMENT A
(EXPRESSED AS PERCENTAGES OF TOTAL NITROGEN)
NON-PROTEIN NITROGEN MINUS AMMONIA
RESIDUAL NON-PROTEIN N
NON-PROTEIN N
PURINE NITROGEN
CREATINE + CREATININE N
AMMONIA NITROGEN
Plotted One-Half the Scale of the Other Quantities
Plotted Ten Times the Scale of the Other Quantities
PERCENT OF TOTAL NITROGEN
50
40
30
20
10
0
START OF EXPERIMENT
2
3
6
7
8
9
10
DAYS

FIG. 1.

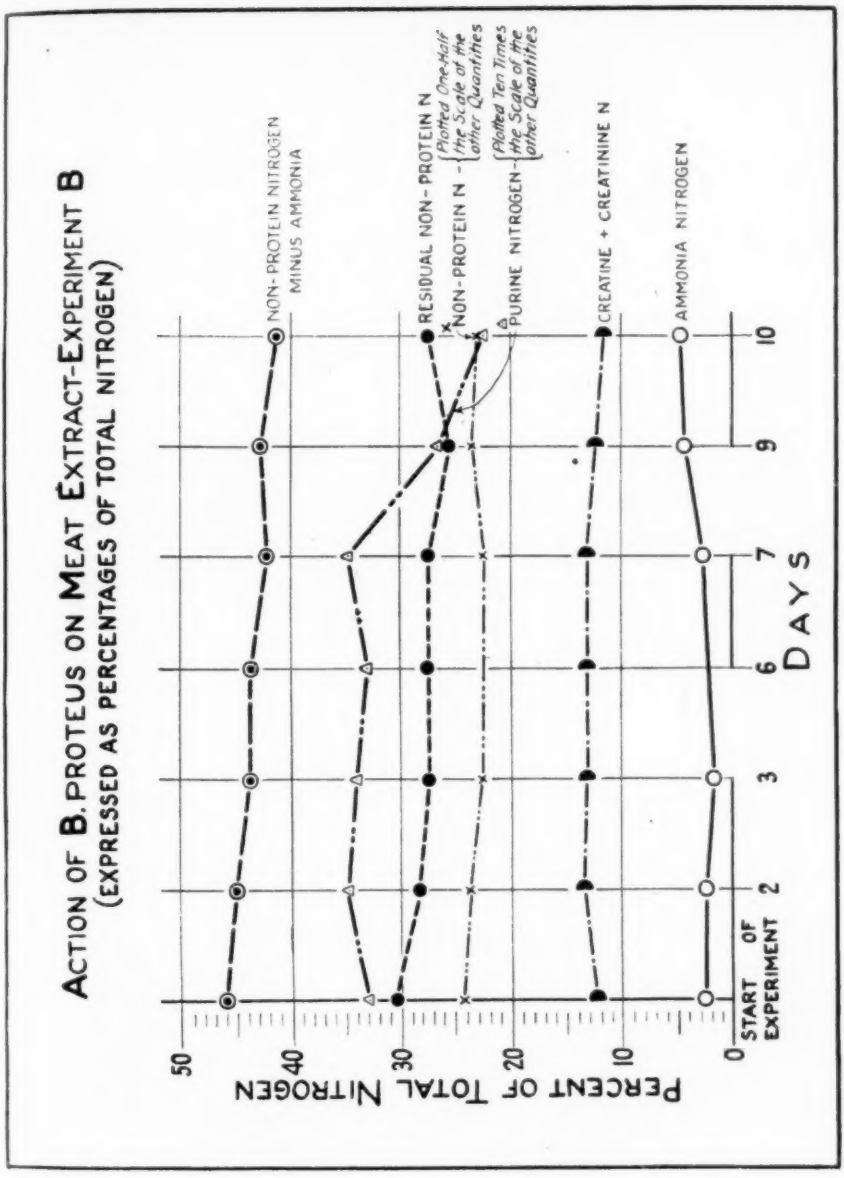

ACTION OF B. PROTEUS ON MEAT EXTRACT-EXPERIMENT B
(EXPRESSED AS PERCENTAGES OF TOTAL NITROGEN)
PERCENT OF TOTAL NITROGEN
50
40
30
20
10
0
START OF EXPERIMENT
2
3
6
7
9
10
DAYS
NON-PROTEIN NITROGEN MINUS AMMONIA
RESIDUAL NON-PROTEIN N
NON-PROTEIN N - (Plotted One-Half the Scale of the other Quantities)
PURINE NITROGEN - (Plotted Ten Times the Scale of the other Quantities)
CREATINE + CREATININE N
AMMONIA NITROGEN

FIG. 2.

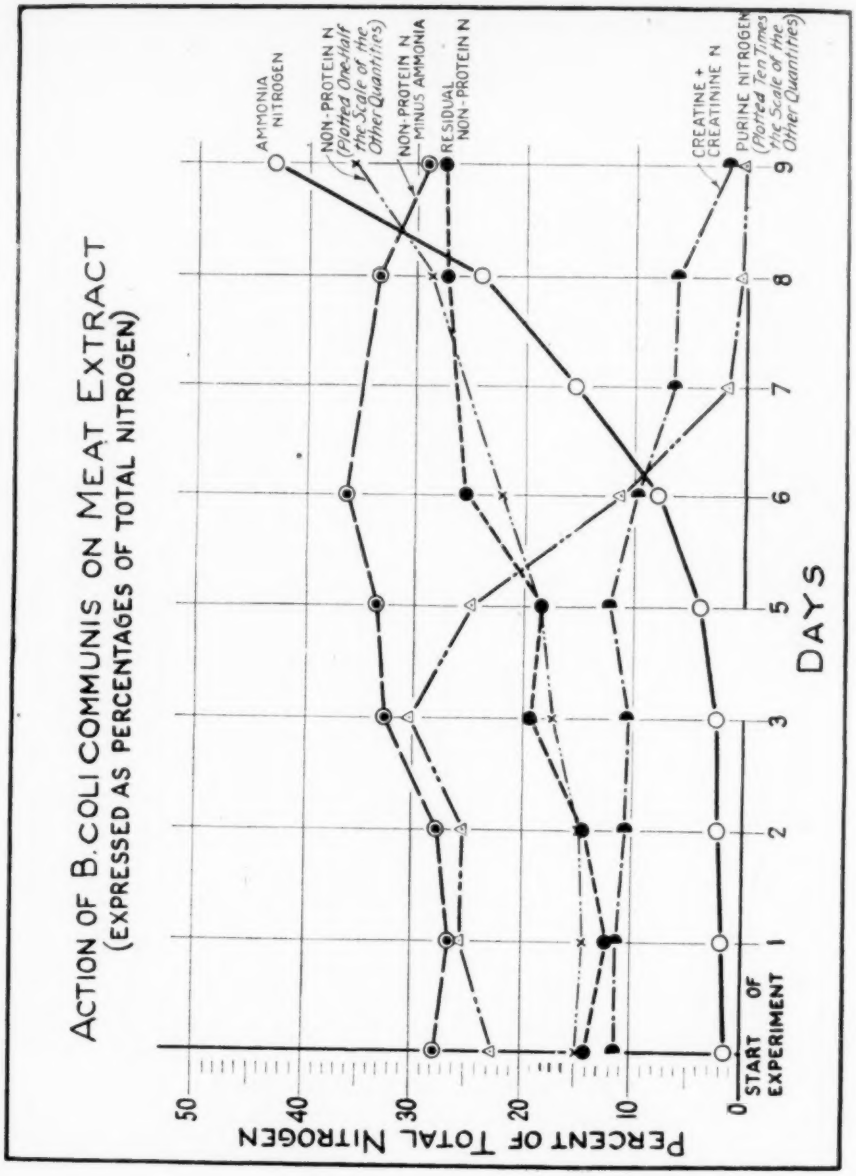

ACTION OF B. COLI COMMUNIS ON MEAT EXTRACT
(EXPRESSED AS PERCENTAGES OF TOTAL NITROGEN)
PERCENT OF TOTAL NITROGEN
50
40
30
20
10
0
START OF EXPERIMENT
1
2
3
5
6
7
8
9
DAYS
AMMONIA NITROGEN
NON-PROTEIN N (Plotted One-half the Scale of the Other Quantities)
NON-PROTEIN N MINUS AMMONIA
RESIDUAL NON-PROTEIN N
CREATINE + CREATININE N
PURINE NITROGEN (Plotted Ten Times the Scale of the Other Quantities)

Fig. 3

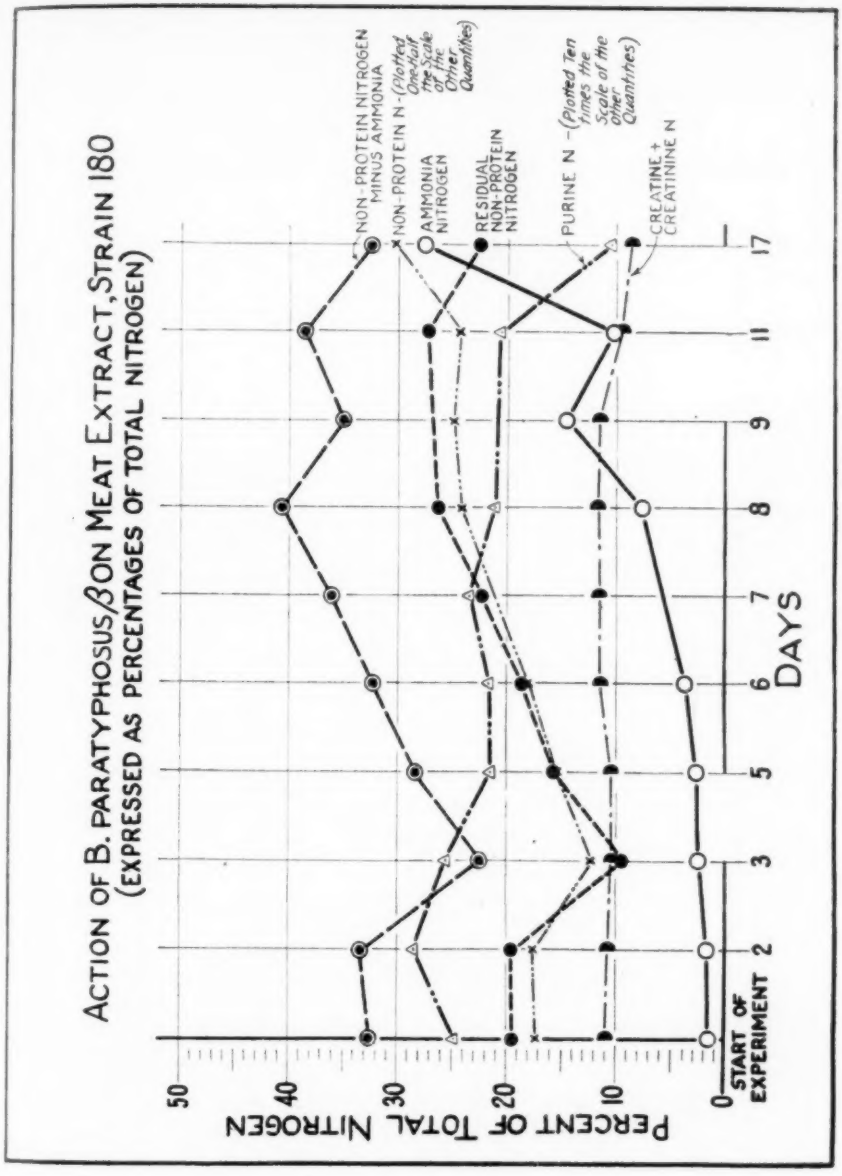

ACTION OF B. PARATYPHOSUS/BON MEAT EXTRACT, STRAIN 180
(EXPRESSED AS PERCENTAGES OF TOTAL NITROGEN)
PERCENT OF TOTAL NITROGEN
50
40
30
20
10
0
START OF EXPERIMENT
2
3
5
6
7
8
9
11
17
DAYS
NON-PROTEIN NITROGEN MINUS AMMONIA
NON-PROTEIN N - (Plotted One-Half the Scale of the Other Quantities)
AMMONIA NITROGEN
RESIDUAL NON-PROTEIN NITROGEN
PURINE N - (Plotted Ten Times the Scale of the other Quantities)
CREATINE + CREATININE N

FIG. 4.

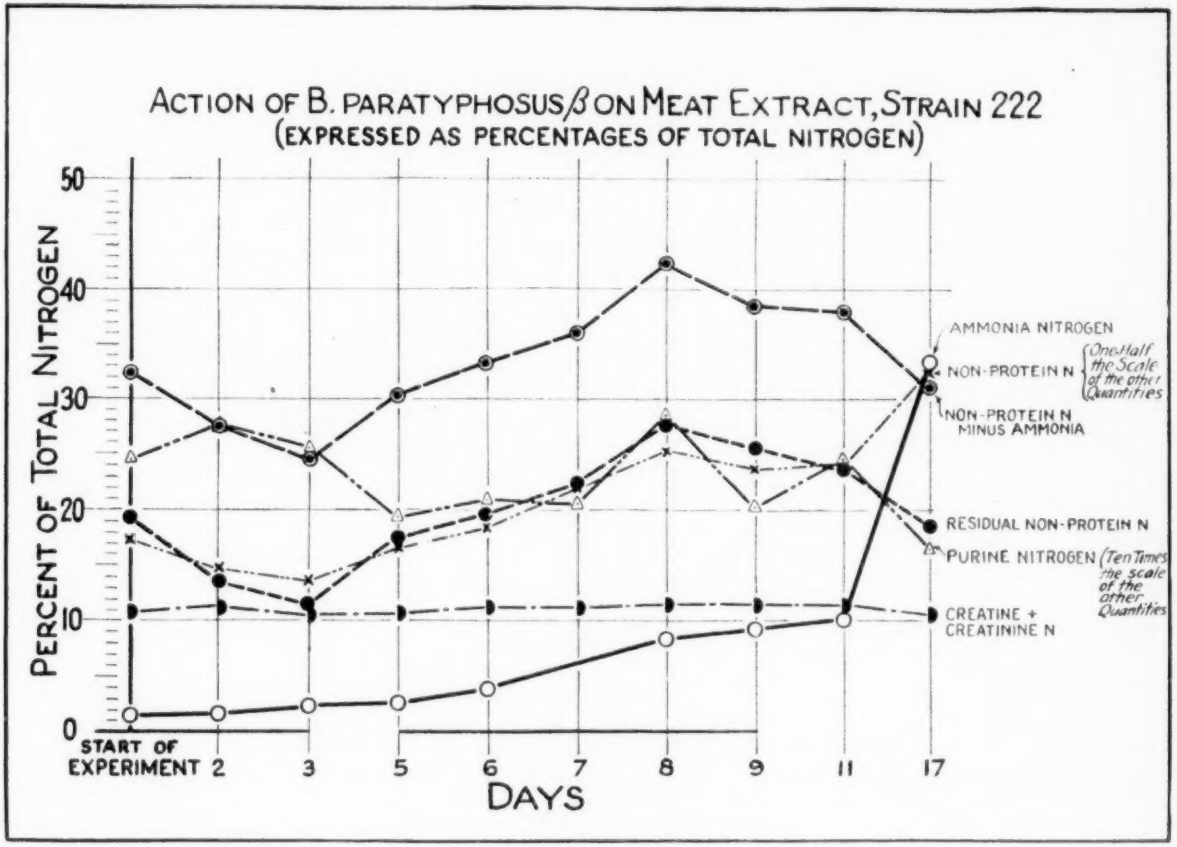

ACTION OF B. PARATYPHOSUS β ON MEAT EXTRACT, STRAIN 222
(EXPRESSED AS PERCENTAGES OF TOTAL NITROGEN)
PERCENT OF TOTAL NITROGEN
50
40
30
20
10
0
START OF EXPERIMENT
2
3
5
6
7
8
9
11
17
DAYS
AMMONIA NITROGEN
NON-PROTEIN N
(One-Half the Scale of the other Quantities)
NON-PROTEIN N MINUS AMMONIA
RESIDUAL NON-PROTEIN N
PURINE NITROGEN
(Ten Times the scale of the other Quantities)
CREATINE + CREATININE N

FIG. 5

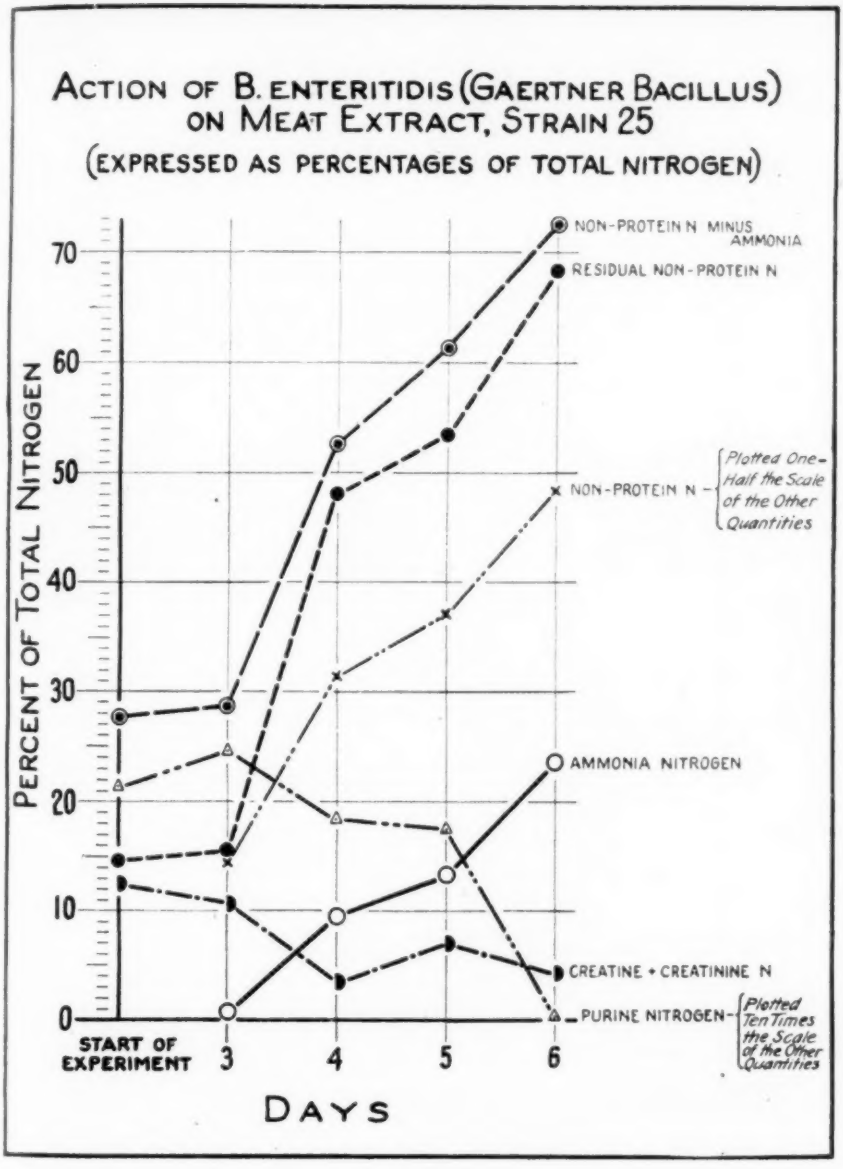

ACTION OF B. ENTERITIDIS (GAERTNER BACILLUS) ON MEAT EXTRACT, STRAIN 25
(EXPRESSED AS PERCENTAGES OF TOTAL NITROGEN)
PERCENT OF TOTAL NITROGEN
70
60
50
40
30
20
10
0
START OF EXPERIMENT
3
4
5
6
DAYS
NON-PROTEIN N MINUS AMMONIA
RESIDUAL NON-PROTEIN N
NON-PROTEIN N
Plotted One-Half the Scale of the Other Quantities
AMMONIA NITROGEN
CREATINE + CREATININE N
PURINE NITROGEN
Plotted Ten Times the Scale of the Other Quantities

FIG. 6

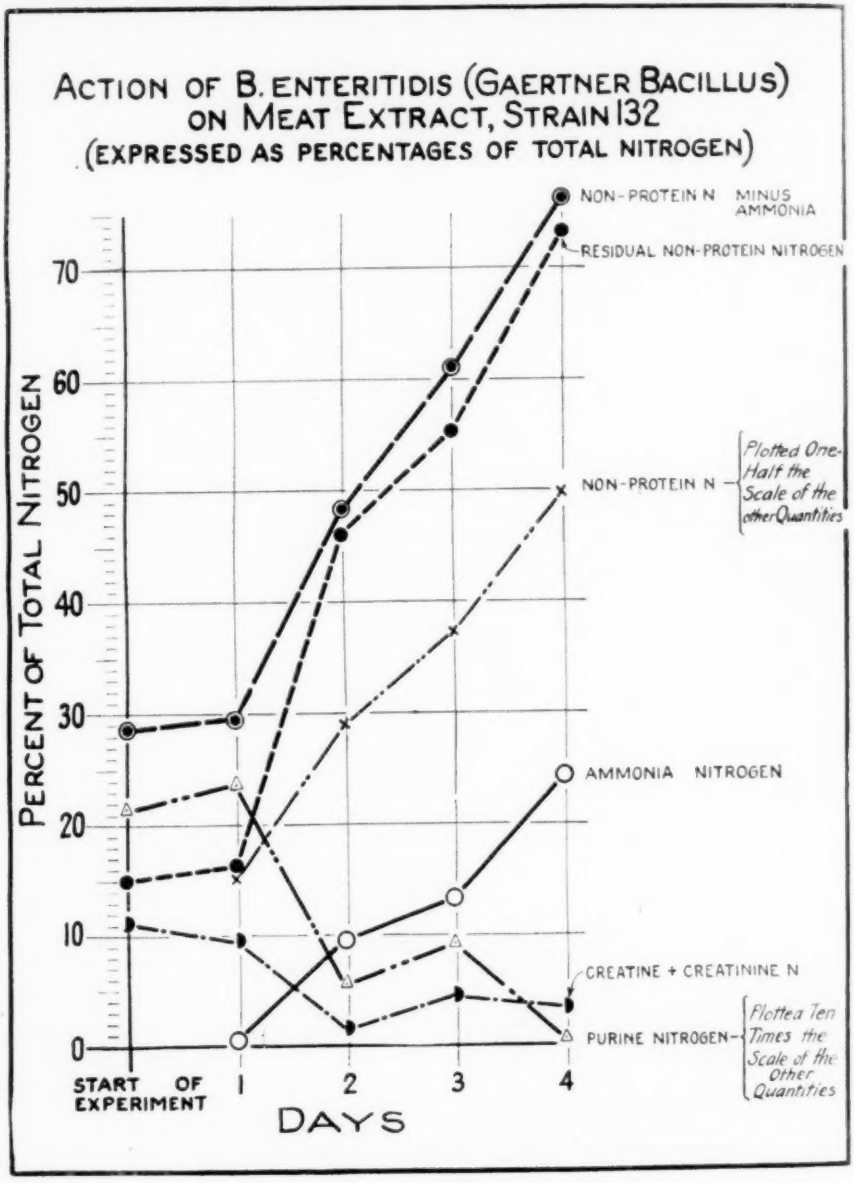

ACTION OF B. ENTERITIDIS (GAERTNER BACILLUS) ON MEAT EXTRACT, STRAIN 132
(EXPRESSED AS PERCENTAGES OF TOTAL NITROGEN)
NON-PROTEIN N MINUS AMMONIA
RESIDUAL NON-PROTEIN NITROGEN
NON-PROTEIN N
Plotted One-Half the Scale of the other Quantities
AMMONIA NITROGEN
CREATINE + CREATININE N
PURINE NITROGEN
Plotted Ten Times the Scale of the other Quantities
PERCENT OF TOTAL NITROGEN
70
60
50
40
30
20
10
0
START OF EXPERIMENT
1
2
3
4
DAYS

FIG. 7.

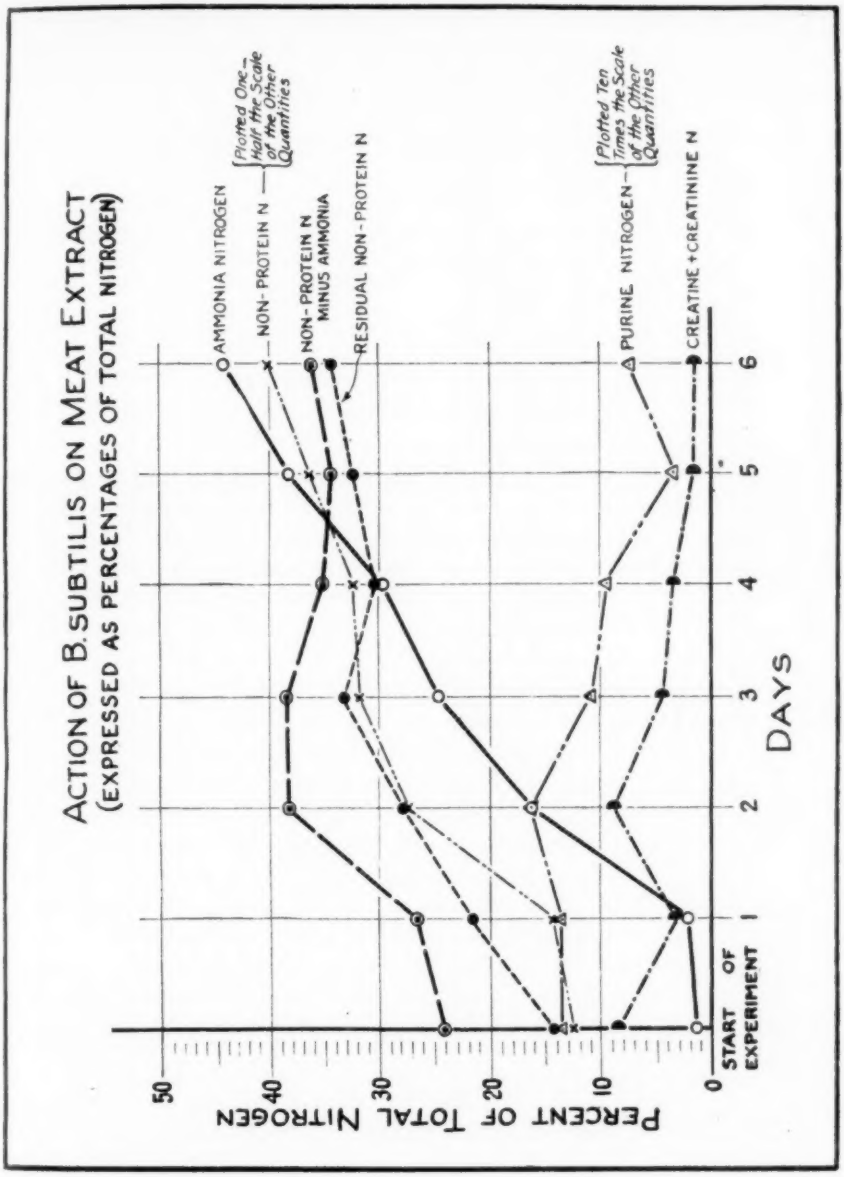
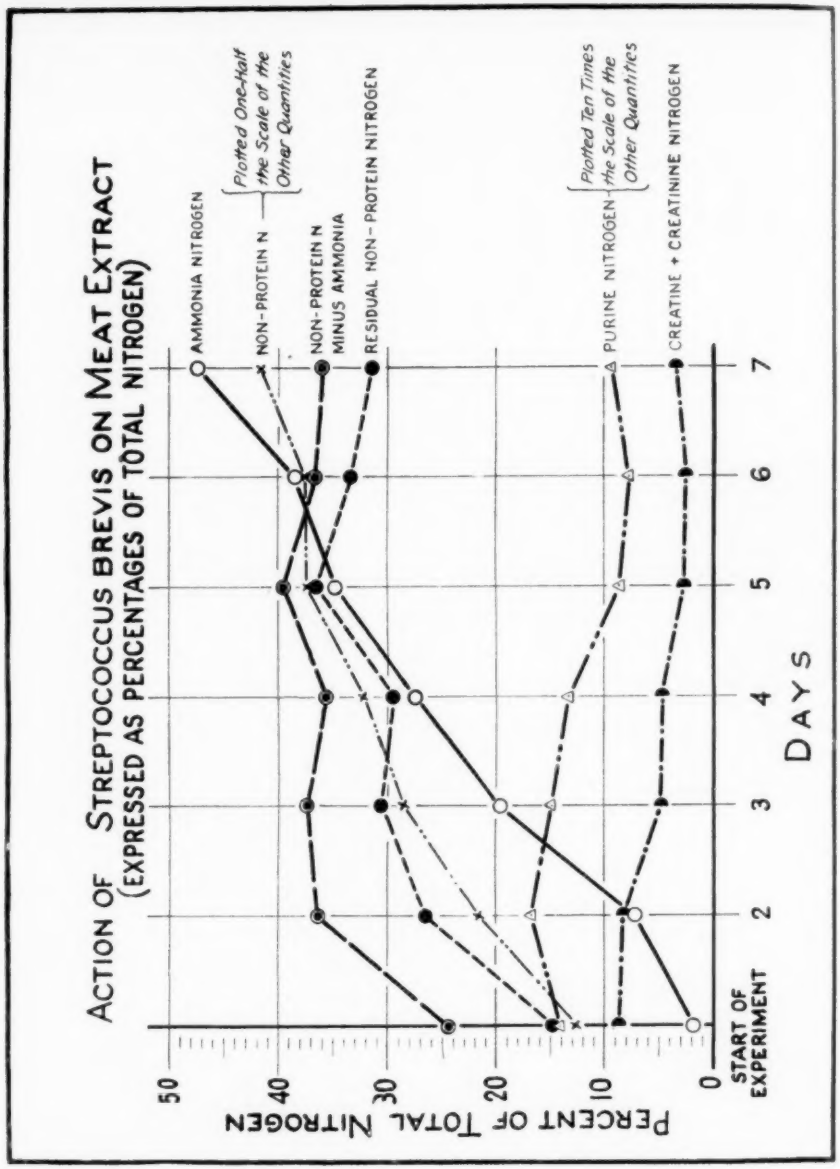

ACTION OF B. SUBTILIS ON MEAT EXTRACT
(EXPRESSED AS PERCENTAGES OF TOTAL NITROGEN)
AMMONIA NITROGEN
NON-PROTEIN N
(Plotted One-Half the Scale of the Other Quantities)
NON-PROTEIN N MINUS AMMONIA
RESIDUAL NON-PROTEIN N
PURINE NITROGEN
(Plotted Ten Times the Scale of the Other Quantities)
CREATINE + CREATININE N
PERCENT OF TOTAL NITROGEN
50
40
30
20
10
0
START OF EXPERIMENT
1
2
3
4
5
6
DAYS

FIG. 8.


ACTION OF STREPTOCOCCUS BREVIS ON MEAT EXTRACT
(EXPRESSED AS PERCENTAGES OF TOTAL NITROGEN)
AMMONIA NITROGEN
NON-PROTEIN N
Plotted One-Half the Scale of the Other Quantities
NON-PROTEIN N MINUS AMMONIA
RESIDUAL NON-PROTEIN NITROGEN
PURINE NITROGEN
Plotted Ten Times the Scale of the Other Quantities
CREATINE + CREATININE NITROGEN
PERCENT OF TOTAL NITROGEN
50
40
30
20
10
0
START OF EXPERIMENT
2
3
4
5
6
7
DAYS

AMMONIA TEST FOR MEAT SPOILAGE.

By K. GEORGE FALK and GRACE McGUIRE.

(From the Harriman Research Laboratory, The Roosevelt Hospital, New York, in Cooperation with the Division of Food and Nutrition, Medical Department, U. S. Army.)

(Received for publication, February 21, 1919.)

In the chapter on "Meat and meat products" in Allen's Commercial Organic Analysis,[1] Richardson stated in reviewing various tests that the estimation of the "nitrogen in ammonium salts, together with other substances easily decomposed by means of weak alkalies, probably affords the best available chemical methods at the present time for the detection of decomposition in flesh foods." Attention was directed to the difficulty of carrying out the methods because of the possibility of decomposition brought about by the reagents used. Ottolenghi, on the other hand, favored the determination of the amount of amino-acid as an index of the beginning of active decomposition.[2] Hoagland and associates[3] found that the increase in amino nitrogen afforded the best measure of the extent of autolysis in cold-stored meats. The behavior of the ammoniacal nitrogen was much the same, but was not considered to be so good an index of the extent of the autolysis.

In the meat studies, some of the results of which were published in the preceding paper, the striking common factor in the decomposition of meat at room temperatures in the form of broth, brought about by a number of definite strains of bacteria, was the marked increase in the ammonia content, especially significant when calculated as percentages of total nitrogen or of non-protein nitrogen. Some experiments on amino-acid determinations showed

[1] Richardson, W. D., in Allen, A. H., Commercial organic analysis, Philadelphia, 4th edition, 1914, viii, 313–16.

[2] Ottolenghi, D., *Z. Untersuch. Nahrungs-u. Genussmittel*, 1913, xxvi, 728.

[3] Hoagland, R., McBryde, C. N., and Powick, W. C., *U. S. Dept. Agric., Bull. 433*, 1917.

such small changes under the conditions used that this method was abandoned.

In attempting to find a chemical test for meat which would indicate when it was on the verge of being unfit to eat, the meaning of unfitness must be defined. Previous work on this question[4] indicates that meat is not poisonous as food when thoroughly cooked if the animal was not infected before death. If the animal was infected when alive, the meat may contain heat-stable toxins. The test for unfitness for meat as purchased in this and other countries having suitable food laws must therefore be based upon its palatability when cooked or upon its flavor, odor, and appearance when uncooked. These standards will vary to some extent in different localities and with different individuals.

From the results of previous work, the ammonia content of meat undergoing decomposition under various conditions was chosen as the test and an attempt was made to find an approximate limit to the ammonia content when the meat would be considered unfit for food.

The difficulties in determining ammonia can be overcome by using the permutit method of Folin and Bell[5] in which ammonia is extracted from a solution by specially prepared permutit (an aluminium silicate, zeolite). This ammonia is then liberated by treatment with alkali and determined by Nesslerization. The method eliminates the possible decomposition by alkali of the protein or other material present and gives results more truly representative of the ammonia in the meat present as such and as ammonium salts.

A number of series of determinations with the aeration and the permutit methods with fresh and decomposed meats gave similar results for the ammonia content, provided certain conditions were adhered to with the former. With 2 to 4 gm. of ground meat and 10 cc. of water, 1.25 cc. of 10 per cent NaOH solution was used, and aeration was continued for 2 hours. Caprylic alcohol was used to prevent foaming, 0.01 N sulfuric acid to absorb the ammonia, and 0.01 N sodium hydroxide for titrating, with the mixture of methyl red and methylene blue as indicator. Instead of sodium hydroxide, 10 cc. of 20 per cent sodium carbonate solu-

[4] Greenwald, I., unpublished data.

[5] Folin, O., and Bell, R. D., *J. Biol. Chem.*, 1917, xxix, 329.

tion may be used, but aeration must then be continued for 3 to 4 hours. For the permutit method, 5 gm. of ground meat (beef) were treated with 60 cc. of water and 20 cc. of alumina cream in a 100 cc. volumetric flask for 1 hour, made up to the mark, filtered, and the ammonia was determined in the filtrate as described by Folin and Bell.

The results obtained with meat (beef) kept at room temperature and at low temperature showed marked differences. With the former, bacterial growth was rapid, especially at the higher temperatures, and the meat was soon unfit for use, in some cases after 24 hours. With the latter, there was considerable growth of mold with little bacterial growth. After trimming off the mold, the meat was still suitable for use after standing for 3 or 4 weeks at a temperature varying between 0° and 5°. With still lower temperatures, the meat could be used after much longer periods.

The ammonia content for the two forms of spoiled meat differed greatly. The fresh meat (beef within 24 hours of slaughter and chilled) contained between 0.03 and 0.10 mg. of ammonia N per gm. of meat. (The results will be given in these terms; the changes in moisture content of the meat were too small to affect the results.)

When allowed to decompose at room temperature (15–25°), the results of a large number of determinations showed that the meat became unsuitable for food when the ammonia nitrogen content reached 0.3 to 0.4 mg. per gm. of meat. Below this, the meat could still be used.

At low temperatures, the ammonia content was much higher before the meat was unsuitable to eat. The results varied considerably because of the variations in temperature and the previous condition and treatment of the meat, but the ammonia nitrogen content was over 1.0 mg. per gm. of meat in every case before the meat was unfit for use and in some cases rose to 3.0 mg. before the meat had to be discarded.

The interpretation of these results, especially in connection with the work of Hoagland and others, is simple. Bacterial growth at room temperature is comparatively rapid and the meat becomes unfit for food even with a low ammonia content. At low temperatures, bacterial growth is slow, but autolysis proceeds so that the cleavage products, such as ammonia and compounds rich in amino nitrogen, increase greatly without the formation of those products

whose odor, appearance, and general flavor make the food unsuitable for use. If the meat is kept cold first, autolysis proceeding, and then is brought to room temperature, decomposition would be much more rapid because of the simpler products formed by autolysis, which would serve as nutriment for bacteria and greatly increase their growth. This may be one reason, in addition to the physical effects of the breakdown of cell walls by freezing, for the more rapid decomposition of meat which has been in cold storage for some time.

The authors wish to express their thanks for the aid given by Miss Helen R. Downes and Mr. Robert P. Greene in carrying out the large number of analyses necessary in this work.

A SIMPLIFICATION OF THE McLEAN-VAN SLYKE METHOD FOR DETERMINATION OF PLASMA CHLORIDES.

By DONALD D. VAN SLYKE and JOHN J. DONLEAVY.

(*From the Hospital of The Rockefeller Institute for Medical Research.*)

(Received for publication, February 19, 1919.)

The method as originally published[1] required for each determination three filtrations as a preliminary to the titration; one after coagulation of the proteins with heat and magnesium sulfate, a second after treatment with charcoal to remove the last traces of protein, and a third after precipitation of the Cl with standard silver nitrate.

We have found that the three filtrations can be condensed into one by addition of picric acid to the standard silver nitrate. The technique consequently involves only one filtration and the measurement of an aliquot part of the filtrate for titration.

Rappleye[2] has recently published a method for plasma chloride determination, based on the Volhard titration, involving a technique as simple as ours, and in fact practically identical, *viz.* a precipitation with silver nitrate and titration of the filtrate, the difference being that Rappleye titrates the excess silver with sulfocyanate instead of iodide. We have obtained entirely accurate results with Rappleye's method. The choice between it and the iodide titration, therefore, depends upon individual preference.

Solutions.—The standard silver nitrate solution (Solution I, p. 363 of the original paper) is made up to contain, in addition to the silver nitrate and nitric acid, 7.5 gm. of picric acid per liter. This solution precipitates both proteins and chlorides simultaneously.[3]

[1] McLean, F. C., and Van Slyke, D. D., *J. Biol. Chem.*, 1915, xxi, 361.

[2] Rappleye, W. C., *J. Biol. Chem.*, 1918, xxxv, 509.

[3] The silver nitrate solution contains per liter 5.812 gm. of pure fused $AgNO_3$, 250 cc. of HNO_3 (specific gravity 1.42), and 7.5 gm. of picric acid.

The starch-citrate-nitrite solution contains per liter 446 gm. of crystalline sodium citrate ($Na_3C_6H_5O_7 + 5\frac{1}{2}\ H_2O$), 20 gm. of $NaNO_2$, and 2.5

The composition of the indicator solution, containing nitrite, citrate, and starch, is unchanged from that described in the original paper. It may be well to emphasize that when the starch is dissolved the solution must be not merely heated, but *boiled* for several minutes, or it will not give a satisfactory end-point. If starch other than the "soluble" variety is used the boiling should continue for an hour.

The $\frac{M}{58.5}$ KI solution, of which 1 cc. is equivalent to 1 mg. of NaCl, has been replaced by $\frac{M}{73.1}$ KI solution, of which 1 cc. is equivalent to 0.8 mg. of NaCl. The greater dilution of the KI solution is conducive to slightly increased accuracy in the titration; and the fact that it is equivalent to 0.8 mg. of NaCl per cc. simplifies the calculation to a single subtraction (see below). The KI solution is standardized to make 2.5 cc. equivalent to 1 cc. of the $AgNO_3$ solution.

Standardization of the KI Solution.—The solution contains 2.27 gm. of pure KI per liter. It is made up to contain 2.4 gm., and diluted to the extent indicated by a preliminary titration. 5 cc. of the silver nitrate solution, measured with a pipette which has an error not greater than 0.01 cc. (delivers 4.97 gm. of water at 20°), are mixed with 5 cc. of the starch-citrate solution and 5 cc. of water, and iodide is run in from a burette to a permanent blue end-point. The amount required should be 12.65 cc., 12.50 cc. being required to precipitate the standard silver solution, and 0.15 cc. additional to give the end-point (see below under "End-point").

Determination of Chlorides in Plasma.—2 cc. of oxalate or citrate plasma are drawn into a dry pipette calibrated to *contain* 2 ± 0.005 cc. (The pipette must weigh 1.994 ± 0.005 gm. more when filled with water at 20° than when empty and dry.) The plasma is run into a 50 cc. measuring flask half full of water, and the pipette is rinsed by drawing the water up into it twice. 10 cc. of the standard silver nitrate-picric acid solution are added,

gm. of soluble starch. The starch is first dissolved in about 500 cc. of boiling water, to which the citrate and nitrite are then added, the solution being finally made up to 1 liter. 4 cc. of this solution contain sufficient citrate to react with the acid in 1 cc. of HNO_3 of 1.42 specific gravity, the resulting solution having the optimum acidity for production of the blue starch-iodine end-point.

and the mixture is diluted to the 50 cc. mark and shaken at intervals for several minutes, until coagulation is completed. The addition of a drop or two of caprylic alcohol prevents foaming and facilitates coagulation. The solution is passed through a dry, chloride-free filter, the first portion of filtrate being passed through, if necessary, a second time to remove turbidity completely. 20 cc. duplicate portions of the filtrate are measured with a calibrated pipette into 100 cc. Erlenmeyer flasks, 4 cc. of the starch-citrate indicator solution are added to each, and the standard KI is run in from a burette until a permanent blue end-point is obtained.

If it is desirable to use less than 2 cc. of plasma, 1 cc. measured within ± 0.002 cc. may be precipitated with 5 cc. of standard silver solution and diluted to 25 instead of 50 cc., the filtrate yielding only one 20 cc. portion instead of duplicates.

The End-Point.—Only a *permanent* and unmistakable blue is taken as the end-point. If the iodide is run in rapidly near the end of the titration, iodine may be formed more rapidly than the silver nitrate precipitates it, and a false end-point reached which disappears after a few seconds shaking. If, towards the end of the titration, the iodide is added in the usual manner, slowly, the blue shade caused by each drop disappears as soon as the solution is rotated, until the genuine end-point is reached. The latter is permanent, and in fact deepens with time.

The first appearance of the end-point is observed easily against a white background, but even more readily against one which matches the light yellow color of the silver iodide precipitate and the picric acid-tinged solution. We usually perform the titration over a sheet of light yellow paper which lies on the table beneath the burette.

The fact that 0.15 cc. of excess KI solution is required to give the end-point does not detract from the accuracy of the titration, because, with a given volume of solution, the required excess is constant and sharply defined. The end-point is not approached gradually, but appears suddenly on the addition of the last drop of the 0.15 cc. excess of KI. This may be demonstrated on a control solution by mixing 1 cc. of HNO_3 of 1.42 specific gravity with 4 cc. of starch-citrate indicator, diluting up to 25 cc., and titrating with the KI solution. The first 0.10 cc. produces no color at all, but the next drop suddenly causes a definite blue which deepens

rapidly for several seconds. The amount of excess KI required to produce the end-point varies directly as the volume of the solution; consequently it is desirable to keep the volume at the end of the titration within approximately the same limits (25 to 30 cc.) in standardizing as in performing the analyses.

Calculation.—The calculation is very simple when standard solutions of the above concentration are used. The 20 cc. of filtrate used for titration represent 0.8 cc. of plasma, and the unprecipitated portion of an amount of $AgNO_3$ equivalent to 8 mg. of NaCl, or 10 mg. per cc. of plasma. Each cc. of KI used in the titration is equivalent to 1 mg. of NaCl per cc. of plasma. Hence the calculation simplifies to:

$$\left.\begin{array}{l}\text{Mg. NaCl per cc. plasma, or}\\ \text{Gm. “ “ liter “}\end{array}\right\} = 10.15 - \text{cc. KI}$$

The use of 10.15 instead of 10 cc. is due to the fact that 0.15 cc. excess of KI solution is required to give the end-point. If the 0.15 cc. of excess iodide required to give the end-point were neglected in the calculation, the error would be partially eliminated by neglecting it also in the standardization. It is as simple, however, to allow for it in the calculation and thereby eliminate it entirely.

Necessity for Calibrated Apparatus.—As compared with most determinations used in physiological and clinical work, exceptional accuracy is required in the estimation of plasma chlorides, for the reason that the significant figure is not so much the total amount as the relatively small difference between that and the normal “chloride threshold” level of 5.62 gm. of NaCl per liter.[4] Consequently errors of more than 0.10 gm. per liter are undesirable. In order to prevent such errors it is necessary to check the accuracy of all pipettes, burettes, and measuring flasks used by calibration. Glass measuring apparatus, because of slow shrinkage after blowing or error in original calibration, is not, as obtained from the dealers, sufficiently accurate to be used for this determination without being checked.

Comparison of Results with Those of Original Method.—The following parallel determinations on a series of human plasmas by

[4] McLean, F. C., *J. Exp. Med.*, 1915, xxii, 212, 366.

the original McLean-Van Slyke method and the present modification indicate that the results are identical.

Plasma No.	NaCl per liter.	
	McLean-Van Slyke method.	Present modification.
	gm.	*gm.*
1	5.90	5.90
	5.90	5.90
2	6.00	6.05
	6.04	6.02
3	6.00	6.00
	6.00	6.02
4	5.35	5.43
	5.35	5.38
5	5.35	5.40
	5.42	5.40
6	5.55	5.55
	5.50	5.52
7	5.68	5.65
	5.63	5.54
8	6.01	6.01
	6.01	6.03

THE NUTRITIVE VALUE OF THE WHEAT KERNEL AND ITS MILLING PRODUCTS.*

By THOMAS B. OSBORNE and LAFAYETTE B. MENDEL.

With the Cooperation of Edna L. Ferry and Alfred J. Wakeman.

(From the Laboratory of the Connecticut Agricultural Experiment Station and the Sheffield Laboratory of Physiological Chemistry in Yale University, New Haven.)

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INTRODUCTION.

In his review of the progress of physiological chemistry for 1917, Professor Hopkins[1] has remarked:

"The present shortage in the food supply of the world makes important every detail of knowledge concerning human nutrition. Even facts which seem academic need scrutiny, in case at some point or other they may find application in the direction of guidance for economy . . . . . Particularly desirable just now is any scrap of knowledge concerning the cereals. Except in arctic climates, bread and cereals are always important items in the food of mankind, and except where wealth has accumulated and luxury come in its train, they are by far the most important. Circumstances have to be very exceptional indeed when the growing of cereals does not yield an energy supply for the worker at less cost and with less relative effort than any other method of food production. Economic and social factors usually tend to make bread by far the most convenient form in which the cereals can reach the individual consumer. The nations of the West have acquired the habit of demanding a well-piled loaf, and for this the special properties of wheat gluten seem necessary. Hence the reliance on wheat in the West."

It is not for mankind alone, however, that wheat has a nutritive importance; for this cereal seed and especially the by-products of its milling are largely used and highly esteemed in feeding domes-

* The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

[1] Hopkins, F. G., *Ann. Rep. on Progress of Chem. 1917, Chem. Soc. London*, 1918, xiv, 179.

tic animals. For reasons which need not be discussed here wheat has been milled more widely and has furnished a larger diversity of milling by-products than any of the other common cereals. These by-products have, in turn, acquired special uses in feeding practice so that their respective nutrient values are also worthy of independent consideration. The debate as to the relative value of the highly milled patent flour in contrast with graham flour or with "whole wheat" flour likewise hinges upon problems in comparative nutrition which have been uniquely emphasized by war time conditions.

Different Parts of the Wheat Kernel.

The wheat kernel consists of three chief parts, each anatomically and chemically differentiated from the others. These are *the embryo,* or *germ,* situated at one end of the kernel as a small, yellow mass, easily distinguished from the rest of the seed; *the endosperm,* which forms much the greater part of the entire kernel, and furnishes food for the embryonic plant when the seed germinates; *the outer seed coats* and underlying layer containing the protein cells which cover the entire seed and protect the embryo and endosperm from damage during the resting period of the seed's existence. Corresponding to the special functions of each of these three parts there are profound differences in the chemical nature of the substances composing them, and consequently wide differences in their nutritive value.

The accompanying tabulation gives the approximate proportion of the above named parts in the average moisture-free wheat kernel containing 2.2 per cent of nitrogen, equal to 12.5 per cent of protein (N $\times$ 5.7); the proportion of the total protein of the seed in these parts; and also the percentage of the total protein in the part itself.

The relative proportion of these parts varies somewhat in different samples of wheat, but in general we believe that these figures fairly represent the average. The amount of endosperm was estimated from the difference between the sum of the other parts and the entire kernel. The amount of embryo was estimated by carefully dissecting out and weighing it. It is impossible to estimate accurately the amount of bran, for it cannot be completely separated from all the other parts of the seed by any method we have

discovered. From the results of milling tests on weighed quantities of wheat[2] and from the data given by Girard[3] we have assumed that 15 per cent is not very far from the truth.

	Part as per cent of seed.	Per cent of seed as nitrogen in part.	Per cent of seed as protein in part (N × 5.7).	Per cent of protein in part.	Per cent of total protein of seed in part.
Endosperm	83.5	1.61	9.17	11.1	73.3
Bran	15.0	0.49	2.80	18.7	22.3
Embryo	1.5	0.10	0.57	36.7	4.4
	100.0	2.20	12.54		100.0

Embryo.

This part of the seed is the center of its physiological activity, to the needs of which the other parts are subservient. Structurally the embryo consists of the rudimentary organs of the embryonic plant which are composed of a mass of small cells of which the nuclei form a far larger proportion than of any other part of the seed. In fact the cell nuclei are so abundant in the embryos that these and yeast have afforded the only material from which nucleic acid has as yet been obtained from vegetable sources.[4] Under proper conditions of moisture and heat, the cells of the embryo begin to divide and chemical processes are set in operation which result in a rapid development of the new tissues of the seedling. The changes in structure, and therefore in composition, which constitute growth imply a high degree of instability in the chemical compounds which compose the greater part of the embryo. Owing to this latter fact chemical investigations of the embryo of wheat are particularly difficult and consequently our knowledge of its constituents is limited.

Frankfurt[5] made a special investigation of the wheat embryo. From the data which he has furnished we have compiled the following statement of its chief constituents.

[2] Wiley, H. W., *U. S. Dept. Agric., Bureau of Chem., Bull. 13*, 1898, pt. 9, 1269.

[3] Girard, A., *Compt. rend. Acad.*, 1897, cxxiv, 876, 926.

[4] Osborne, T. B., and Campbell, G. F., *J. Am. Chem. Soc.*, 1900, xxii, 379.

[5] Frankfurt, S., *Landw. Versuchsstat.*, 1896, xlvii, 449.

Composition of the Wheat Embryo.

	per cent
Carbohydrates soluble in water, including sucrose and raffinose (6.89 per cent)	24.34
"Crude protein" (N = 6.44 per cent × 6.25)*	40.25
Fiber	1.71
Crude fat	13.51
Ash	4.82
Undetermined (chiefly insoluble carbohydrates?)	15.37
	100.00

* We have confirmed Frankfurt's estimate of nitrogen by Kjeldahl determinations made on very pure embryos isolated with great care.

Compared with the other parts of the seed the proportion of soluble carbohydrates is large, about 15 to 18 per cent of the embryo being sugars of which, according to Richardson and Crampton,[6] sucrose is the most abundant, while another, supposed by them to be raffinose and later identified as such,[7] is present in considerable amount. Starch is wholly lacking, as shown by microscopic examination of the isolated embryo.

Richardson and Crampton[6] identified allantoin among the nonprotein nitrogenous compounds and Frankfurt[5] added to these asparagine, choline, and betaine. Later nucleic acid was found to be present in considerable, though undetermined, amount.[4] In view of the fact that the proportion of these nitrogenous constituents is still unknown no accurate statement of the percentage of pure protein in the embryo can be given. It must, however, be decidedly less than the amount of "crude protein" as estimated by multiplying the total nitrogen by the conventional factor.

The proteins of the embryo differ markedly from those of the endosperm, both in their solubility and constitution.[4] Water extracts about 10 per cent of albumin from commercial wheat embryo meal, and sodium chloride solution further extracts about 5 per cent of globulin, whereas only very small amounts of similar proteins are thus extracted from wheat flour (endosperm). It is probable that both albumin and globulin are present in larger proportion in the embryo than the above figures indicate, for the insolu-

[6] Richardson, C., and Crampton, C. A., *Ber. chem. Ges.*, 1886, xix, 1180.
[7] Schulze, E., and Frankfurt, S., *Ber. chem. Ges.*, 1894, xxvii, 64.

ble compounds which these proteins form with nucleic acid doubtless preclude their complete extraction. The proportion of the products of hydrolysis of the albumin, known as leucosin, is similar to that from proteins of many animal products which are commonly used for food, but is strikingly different from that yielded by the proteins of the endosperm of the wheat kernel.[5]

Owing to the difficulties encountered in separating the embryo from the other parts of the kernel it is practically impossible to obtain large quantities free from admixtures. We were therefore compelled to use a commercial preparation, kindly furnished by the Quaker Oats Company. By sorting this material under a lens about 33 per cent, obviously chiefly bran, could be separated, while the embryo tissue which remained still contained not a little endosperm as evidenced by the starch grains.

The following data, obtained by the conventional methods commonly used for analyses of this kind, show the general character of the material which we employed for our feeding experiments. From the nature of the methods employed these figures can at best represent only approximately the proportion of most of the designated groups of constituents.

Composition of Commercial Wheat Embryo Meal.

	per cent
Sugars	7.71
Dextrin	7.50
Starch	18.21
Pentosans, etc.	8.29
Protein (N × 6.25)	31.00
Fiber	2.35
Fat	10.44
Ash	4.91
Undetermined	9.59
	100.00

By carefully grinding a sample of the meal after it had been extracted with ether the friable particles of the embryo were readily reduced to a powder while the more resistant particles of bran and a part of the endosperm were less easily reduced in size. By sifting on suitable sieves three fractions were obtained, the finest containing only a small amount of bran visible under the lens.

[5] Osborne, T. B., and Clapp, S. H., *Am. J. Physiol.*, 1906-07, xvii, 231.

The intermediate fraction, stained with iodine, consisted almost, if not wholly, of particles of endosperm and bran in approximately equal parts, so far as could be visually estimated. The coarsest fraction was chiefly bran to which some endosperm still adhered. Neither of the two coarser fractions appeared to contain appreciable amounts of embryo tissue. Assuming that one-half of the intermediate fraction was bran and that the endosperm in the coarsest fraction was approximately equal to the bran remaining in the finest fraction, we estimate that the original embryo meal before extraction with ether contained about 23.7 per cent of pure bran. From the starch content of the embryo meal we also estimate its content of endosperm at 23.5 per cent and in pure embryo tissue by difference at 52.8 per cent. Although the above method of determining the composition of the commercial embryo meal used for the experiments to be described later permits only a rough estimate, it gives the best idea of the character of this meal that we have been able to obtain.

The following tabulation shows the proportion of nitrogen and protein contained in this embryo meal, dried at 110°.

		Commercial embryo meal contains	
		Nitrogen.	Protein.
	per cent	*per cent*	*per cent*
Endosperm (N = 1.92 per cent)	= 23.5	0.46 (N × 5.70)	2.62
Bran (" = 2.88 " ")	= 23.7	0.68 (" × 6.25)	4.25
Embryo (" = 7.0 " ")	= 52.8	3.70 (" × 6.25)	23.10
Total N in meal		4.84	
Found		4.96	

The nitrogen and protein in this meal were in the following proportions:

	Nitrogen.	Protein
	per cent	*per cent*
In endosperm	9.5 (N × 5.7)	8.7
" bran	14.1 (" × 6.25)	14.2
" embryo	76.4 (" × 6.25)	77.1
	100.0	100.0

Thus although only a little more than one-half of the embryo meal was probably pure embryo, more than three-quarters of the nitrogen belonged to that part of the seed.

Seed Coats or Bran.

Wheat bran is made up of several layers of cells which, when the seed is dry, adhere so firmly to one another that they are removed by the milling process in comparatively large pieces. By sifting and other mechanical means much is obtained nearly free from embryo and endosperm. As the separation is never perfect, however, even the purest commercial bran always contains a little endosperm and possibly traces of embryo.

The most conspicuous layer of cells composing bran is the innermost one which, in the entire seed, lies next to the endosperm. This layer does not belong strictly to the outer seed coats, for it is supposed to be a continuation of the embryo. For the purposes of our present study it must be included with the true seed coats because it is always removed with these latter in the milling process. The cells of this inner layer are thick-walled and contain a large part, perhaps the whole, of the protein of the bran. Unfortunately the thick walls of these cells make it so difficult to extract the proteins from bran that these have never been obtained in sufficient quantity or in suitable condition for determination of their physical or chemical characters. From the little that is known about these proteins, however, it is evident that they are unlike those of the gluten which constitute approximately 80 per cent of the protein of the endosperm. Chemically, as well as structurally, bran differs greatly from the embryo and endosperm and consequently also in its nutritive value. The thick cell walls which enclose so large a part of its nutrients render the digestion of these difficult for man, while cattle and poultry with their type of digestive organs utilize them readily.

As it is practically impossible to prepare large quantities of bran any freer from other parts of the seed than the so called pure bran of commerce, we have used a preparation of the latter for the feeding experiments described in this paper. The approximate proportion of the various groups of constituents contained in this preparation of bran is shown by the following data obtained by the

same conventional method as was employed in analyzing the wheat embryo.

Composition of Commercial Bran, Moisture-Free.

	per cent
Sucrose	1.64
Dextrin	4.19
Starch	13.39
Pentosans, etc.	27.58
Protein (N × 6.25)	17.00
Fiber	9.37
Fat	7.07
Ash	7.24
Undetermined	12.52
	100.00

Careful examination of this sample of bran showed that it was practically free from embryo. Since it contains starch it must consequently contain endosperm, for microscopic examination shows that none of the layers of cells which constitute really pure bran contains starch. The amount of endosperm in our commercial bran can, therefore, be estimated from its starch content at about 17 per cent. On this assumption the above analysis can be corrected to show more nearly the approximate composition of pure bran.

Approximate Composition of Pure Bran, Moisture-Free.

	per cent
Sucrose	1.92
Dextrin	5.03
Starch	0.00
Pentosans, etc.	32.77
Protein (N × 6.25)	18.00
Fiber	11.18
Fat	8.26
Ash	8.61
Undetermined	14.23
	100.00

The proportion of nitrogen and protein in the commercial bran which we used was approximately as follows, calculated moisture-free:

	Commercial bran contains	
	Nitrogen.	Protein.
	per cent	*per cent*
Endosperm (N = 1.96 per cent).....	0.33 (N × 5.7)	1.88
Bran (" = 18.0 " ").....	2.39 (" × 6.25)	14.94
	2.72	16.82

Of the total protein in this preparation 11.2 per cent belonged to endosperm and 88.8 per cent to the pure bran.

Endosperm.

The endosperm forms about 84 per cent of the entire seed, the proportion varying with the plumpness of the grain. This part of the seed furnishes food for the growing embryo. Unlike the constituents of the embryo, those of the endosperm are relatively stable substances, designed by nature to remain unchanged until the germinating embryo draws on them for its first supply of food.

The following figures show the proportion of approximate constituents of the wheat flour which we used.

Composition of Wheat Flour, Moisture-Free.

	per cent
Sucrose	0.24
Dextrin, etc.	3.63
Starch	77.52
Pentosans	1.80
Protein (N × 5.7)	11.44
Fiber	0.34
Fat	1.15
Ash	0.43
Undetermined	3.45
	100.00

In contrast to the other parts of the seed the endosperm is characterized by its very high starch content which, with the protein, equals nearly 89 per cent of the whole. Since most of the protein of the endosperm contains 17.5 per cent of nitrogen we have employed the factor 5.7 in estimating the percentage of protein from the nitrogen content of the flour.

The greater part of the total protein of the wheat kernel is stored in the large cells of the endosperm. Of this about 80 per cent consists of two kinds, gliadin and glutenin. These proteins unite to form the gluten, characteristic of wheat, which is physically and chemically unlike any protein product obtainable from other seeds. The unique physical properties of wheat gluten render flour made from this grain better for making bread than that obtained from the seeds of any other cereal. The tenacious character of wheat gluten when combined with water is such that the carbonic acid liberated by yeast when bread is leavened is so retained within the loaf as to impart to it a special lightness which cannot be secured by using any other flour.

Whole Wheat Kernel.

The proteins of the wheat kernel have been extensively studied in this laboratory[9] and the knowledge thus gained, as well as that obtained in other laboratories, has been critically reviewed in a later publication.[10] The relative proportion of amino-acids supplied by the wheat proteins is quite unlike that furnished by most other food proteins. Thus when hydrolyzed gliadin yields two and one-half times more ammonia, three or four times more glutaminic acid and proline, and only one-third to one-fourth as much basic amino nitrogen as do most food proteins. Furthermore, gliadin yields so little lysine[11] that young animals grow extremely slowly when fed on diets containing gliadin as the sole source of protein unless lysine is added to their food.[12] The products of hydrolysis of gliadin have been studied in this laboratory[8] and more recent data were summarized later.[13]

Glutenin yields relatively large proportions of ammonia and glutaminic acid, though less than gliadin yields; also more basic amino-acids[8] and enough lysine to permit of good growth when animals are fed on diets containing sufficient glutenin as the only

[9] Osborne, T. B., and Voorhees, C. G., *Am. Chem. J.*, 1893, xv, 392.

[10] Osborne, T. B., *Carnegie Institution of Washington, Publication 84*, 1907.

[11] Osborne, T. B., Van Slyke, D. D., Leavenworth, C. S., and Vinograd, M., *J. Biol. Chem.*, 1915, xxii, 259.

[12] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1914, xvii, 325.

[13] Osborne, T. B., and Guest, H. H., *J. Biol. Chem.*, 1911, ix, 425.

protein.[14] All of the amino-acid deficiencies of gliadin are, to some extent, supplemented by glutenin, so that wheat gluten affords a mixture of proteins on which young animals grow fairly well.[15]

Besides gliadin and glutenin the wheat endosperm (wheat flour) yields a small proportion of globulin, albumin, and proteose.[9] Since similar proteins have been found in the embryo[4] possibly their presence in flour may be due in part to an incomplete separation of the embryo in milling.

The relatively large proportion of ammonia, glutaminic acid, and proline yielded by the proteins of wheat flour compared with that yielded by proteins from other sources commonly used for food must have a marked influence on the nutritive value of white flour. Evidence of this is indicated by the experiments described in this paper, but further studies of this question should be made under more rigorously controlled conditions.

The accompanying figures give the proportion of the proximate constituents of the whole wheat used for most of the experiments described in this paper, as determined by an analysis which we have made according to the conventional methods employed for the analysis of the other parts of the kernel. The latter are here reproduced so that the very marked differences in their chemical composition may be the more readily appreciated.

	Whole wheat.	Endosperm.	Bran.	Embryo.
	per cent	*per cent*	*per cent*	*per cent*
Sucrose	0.61	0.24	1.92	24.34
Dextrin, etc.	2.01	3.63	5.03	
Starch	63.80	77.52	0.00	0.00
Pentosans, etc.	6.27	1.80	32.77	?
"Crude protein"	11.25	11.17	17.60	40.25
Fiber	1.75	0.34	11.18	1.71
Fat	2.18	1.15	8.26	13.51
Ash	2.03	0.43	8.61	4.82
Undetermined	10.10	3.72	14.63	15.37
	100.00	100.00	100.00	100.00

[14] Osborne, T. B., and Mendel, L. B., *Carnegie Institution of Washington, Publication 156*, pt. 2, 1911.

[15] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1917, xxix, 69.

While we recognize that the above analyses may be fairly criticized on many grounds they serve to illustrate the very wide differences in the proportion of the various types of nutrients in the different parts of the wheat kernel into which it is separated by the miller.

Milling.

In the old fashioned process of milling, the entire kernel was ground between stones and the flour obtained by sifting through reels covered with fine-meshed silk cloth. Owing to the tough fibrous structure of the outer coats of the seed (bran) the greater part of this was removed, but a portion of the bran together with most of the embryo, or germ, passed through the sieve and was mixed with the flour. The product of this old fashioned milling was essentially the so called "entire wheat flour." When the bolting process was omitted and the coarse bran left in the ground product it has been known as graham flour.

Owing to the presence of the germ, flour made in this crude way cannot be kept long; and inasmuch as the bread made from such flour is dark in color the method of grinding wheat with stones has been almost entirely superseded by the Hungarian or roller mill process. The purpose of this is to separate the kernel into the three parts into which it is anatomically differentiated, namely the outer seed coats or bran, the embryo or germ, and the endosperm or berry of the grain. To accomplish this the grain is reduced to particles of successively smaller size; and by interposing suitable siftings on cloths of carefully chosen mesh the bran and germ are very completely separated from the flour. In carrying out this process about 30 per cent of the kernel is obtained, mostly as mixtures of all parts of the seed which cannot profitably be further separated.[16] These mixtures are chiefly used for feeding domestic animals. It commonly has been assumed that high grade flour, from which bran and germ have been so completely removed, is less nutritious than that made by the older process; but little convincing evidence as to the justification for this assumption has been presented until recently.

[16] A scheme representing the successive steps in the flour milling process has been published by Voegtlin, C., and Myers, C. N., *Public Health Rep.*, 1918, xxxiii, 911.

Nutritive Value of the Wheat Kernel and Its Parts.

Literature.—Although the nutritive value of the wheat kernel has been the subject of extensive investigations they have related almost wholly to the digestibility and utilization of its various parts and constituents. Since these studies have at present but little bearing on the subjects discussed in this paper we will limit our review to the more recent investigations directly concerned with the problems we have studied.

The *wheat kernel as a whole*, according to McCollum, Simmonds, and Pitz,[17] contains sufficient water-soluble vitamine as well as protein of suitable quality to promote nearly normal growth in rats, provided the grain is supplemented with adequate salts and fat-soluble vitamine. In their experiments, however, reproduction was not normal until the protein of the ration was modified by the addition of casein.

The *wheat embryo*, according to the same authors,[18] contains proteins of very superior quality, and an abundance of the water-soluble vitamine. The content of inorganic elements is inadequate and the amount of fat-soluble vitamine is insufficient to promote adequate growth when the embryo is depended upon to furnish these essential constituents of the ration. They also believe that the germ contains something which is distinctly toxic to animals, and can be partially removed by extraction with ether. With reference to this toxicity of wheat, Hopkins[19] says:

"It is claimed that part of the symptoms displayed by animals on an exclusive wheat dietary containing the whole grain are due to a toxic factor. I am not quite sure that the evidence for this view is yet sufficient. Still further observations of a quantitative sort involving the adjustment of deficiencies seem first called for. Nevertheless, an oil with definitely toxic properties has been extracted from the wheat embryo."

Likewise Voegtlin and Myers[20] assert that "no evidence of a toxic action of a whole wheat diet was obtained in the experi-

[17] McCollum, E. V., Simmonds, N., and Pitz, W., *J. Biol. Chem.*, 1916–17, xxviii, 211. McCollum, E. V., and Davis, M., *ibid.*, 1915, xxi, 615.

[18] McCollum, E. V., Simmonds, N., and Pitz, W., *J. Biol. Chem.*, 1916, xxv, 105.

[19] Hopkins, F. G., *Ann. Rep. on Progress of Chem. 1917, Chem. Soc. London*, 1918, xiv, 180.

[20] Voegtlin, C., and Myers, C. N., *Public Health Rep.*, 1918, xxxiii, 843.

ments on squabs which were fed on whole wheat meal, supplemented by a suitable salt mixture." These investigators, speaking of the growth-promoting properties of foods derived from corn and wheat, arrived at the following conclusions:

"The 'highly milled' products are, without exception, inferior in dietary value, as regards growth, to foods prepared from the whole grain. It is rather surprising that such delicate organs as the gastro-intestinal tract of young mice can tolerate a diet containing a large amount of bran. This fact, however, does not necessarily mean that it is advantageous to include the bran in foods intended for human nutrition. On the contrary, the experiences with 'war bread' would rather indicate that persons with delicate digestion are subject to temporary digestive disturbances as a result of a change from 'white' bread to bread containing a considerable percentage of bran ('war bread'). On the other hand, from the standpoint of dietary completeness, a bread including all of the grain, with the exception of the superficial cellulose layer, is undoubtedly superior to the so-called white bread, made from 'highly milled' flour, and would not possess the above-mentioned objectionable features. The 'white' bread used in these experiments was not adequate for maintaining normal growth, in spite of the fact that it was prepared with some evaporated milk and yeast. The most significant defect of 'white' flour is the deficiency in antineuritic and fat-soluble vitamine; it is also deficient in adequate protein and inorganic salts. A wheat flour, containing a considerable part of the germ and superficial layers of the grain, supports growth of mice and pigeons especially well when supplemented with inorganic salts. The same is true of 'whole wheat' bread."

The nutritive value of the endosperm proteins, gliadin and glutenin, has been the subject of earlier reports by us.[21] On otherwise adequate diets in which glutenin represents practically the sole source of protein, growth can readily be induced; not so, however, when gliadin, low in its yield of lysine, is similarly employed.

From the publications cited it appears that the water-soluble vitamine is chiefly, if not wholly, located in the embryo; that the proteins of the entire kernel, eaten in sufficient quantity, are adequate for normal growth; that the proteins of the embryo are superior to those of the endosperm in promoting growth; that the proteins of the endosperm are probably inferior in this respect to those of the entire kernel; and that better growth can be made when the proteins of the endosperm are supplemented by casein

[21] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1912, xii, 473.

than when fed as the sole source of protein. However, owing to lack of data concerning food intakes only very general conclusions respecting the relative content in water-soluble vitamine, or of the nutritive value of the proteins of the endosperm and embryo of the wheat kernel, can be formed. Furthermore practically no information is available regarding wheat bran in respect to these nutritive factors.

Accordingly we have undertaken an extensive series of experiments on rats for the purpose of comparing quantitatively the entire wheat kernel and its milling products: wheat flour, wheat embryo, and wheat bran, with respect to the nutritive value of their proteins, and their content of water-soluble vitamine, both for the maintenance of adult rats and the growth of young ones.

EXPERIMENTAL.

Entire Wheat Kernel as a Source of Protein.

Maintenance Experiments.—Special difficulties are encountered in determining the protein minimum for maintenance, some of which we have discussed in a former paper.[22] The additional experience gained in making the present experiments still further emphasizes these difficulties and has raised new questions which must be answered before we can draw very precise conclusions from the results obtained.

An inspection of the charts shows that protein intake and gain or loss of body weight do not run closely parallel, periods of slight gain in weight often being followed by a similar loss, although somewhat more protein was eaten in the latter period. Many cases where the converse is true are also to be observed. Occasional variations of 5 to 10 gm. in the weight of adult rats are of no importance in interpreting the results of these experiments, for such are to be attributed to more or less food, water, or feces in the animal at the time of weighing. A continued gain or loss, however, extending over several periods of weighing may be considered to represent a real change in the weight of the animal. The present series of experiments also has indicated that the vitamine concentration of the diet may have influence on the

[22] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1915, xxii, 241.

CHART I. The figures on the curves, indicating mg. of protein eaten weekly per gm. of body weight, show that when *whole wheat* furnishes all of the protein and water-soluble vitamine of an otherwise adequate dietary rats are not maintained unless their average weekly protein intake is above 20 mg. per gm. of body weight.

The composition of the food mixtures was as follows:

	5 per cent protein (N × 5.7).	6.5 per cent protein (N × 5.7).	7 per cent protein (N × 5.7).	10 per cent protein (N × 5.7).
	per cent	*per cent*	*per cent*	*per cent*
Whole wheat	44	60	63	92
Salt mixture	3	3	3	3
Starch	28	12	9	
Butter fat	9	9	9	5
Lard	16	16	16	

The composition of the salt mixture was:

	gm.		*gm.*
$CaCO_3$	134.8	Citric acid + H_2O	111.1
$MgCO_3$	24.2	Fe citrate $1\frac{1}{2}$ H_2O	6.34
Na_2CO_3	34.2	KI	0.020
K_2CO_3	141.3	$MnSO_4$	0.079
H_3PO_4	103.2	NaF	0.248
HCl	53.4	$K_2Al_2(SO_4)_4$	0.0245
H_2SO_4	9.2		

The chemicals used were analyzed and allowance was made for moisture, etc. The acids were mixed and the carbonates and ferric citrate added to them. The traces of KI, $MnSO_4$, NaF, and $K_2Al_2(SO_4)_4$ were added as solutions of known concentrations. The final resulting mixture was evaporated to dryness in a current of air at 90–100°C., and ground to a fine powder.

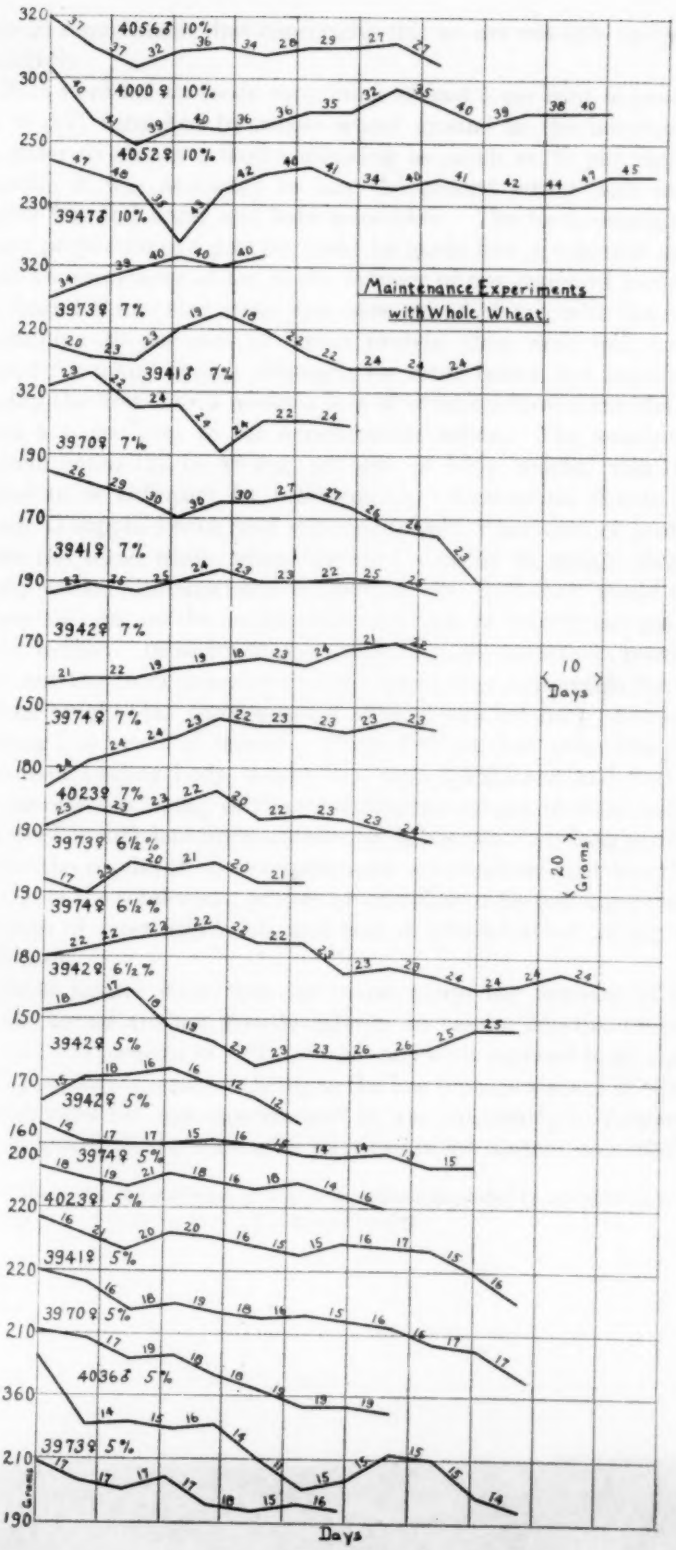

Maintenance Experiments
with Whole Wheat.
40568 10%
40008 10%
40528 10%
39478 10%
39738 7%
39418 7%
39708 7%
39418 7%
39428 7%
39748 7%
40238 7%
39738 6½%
39748 6½%
39428 6½%
39428 5%
39428 5%
39748 5%
40238 5%
39418 5%
39708 5%
40368 5%
39738 5%
10 Days
20 Grams
Grams
Days

CHART I.

protein requirement; but concerning this we are not able to speak positively.

Rats were fed on foods containing 10 and 5 per cent of protein (N × 5.7) furnished by whole wheat ground in the laboratory. In order to obtain a food containing as much as 10 per cent of protein, it was necessary to mix the ground wheat with salts, butter fat, and water and bake into cakes. The foods containing lower proportions of protein could be made into a coherent mass with an abundance of fat in the manner of our standard foods.

Chart I shows that when rats were fed *ad libitum* with the food containing 10 per cent of wheat protein they were well maintained for many weeks, although, for some reason not apparent, during the first 2 or 3 weeks a loss of weight followed the change from a mixed diet to the experimental ration. The amount of protein eaten (27 to 48 mg. per gm. of body weight) was thus found to be sufficient for maintenance. Another set of rats (see Chart I) supplied with food containing only 5 per cent of protein from the same whole wheat declined steadily in weight during many weeks, although they received all the food they would eat. These rats ate on the average about 16 mg. of protein per gm. of body weight. In order to show that the low content in protein, and not the total quantity of food eaten, was responsible for the falling weight, the same amount of food was fed daily, but containing 7 per cent of protein. Chart I shows that under this condition of feeding body weight was well maintained and that an intake of about 23 mg. of the total proteins per gm. of body weight per week is sufficient for maintenance during relatively long periods. From the results of these experiments we conclude that less than 20 mg. of the total wheat protein are insufficient for prolonged maintenance of most adult rats and that in general about 23 mg. are sufficient.

These figures show that for maintenance the proteins of the wheat kernel are not greatly inferior to casein, edestin, or even to the total proteins of milk, but are somewhat superior to gliadin.[22]

Growth Experiments.—Owing to the low protein content of wheat kernel used for our experiments[23] it was impossible to prepare a food comparable to our earlier mixtures in fat content and calorific

[23] Owing to the shortage of wheat we were compelled to use such samples of wheat and flour as we could obtain locally.

value, and still containing more than 8 per cent of protein. Diets with lower contents furnish too little protein for normal growth. Accordingly a food was prepared containing 92 per cent of the whole wheat kernel ground in the laboratory (= ca. 10 per cent protein, N × 5.7) supplemented with salts and only 5 per cent of butter fat. This was mixed with enough water to make a soft dough and fed either in the moist form, or after drying in hard cakes. In some cases, the unground kernels were smeared with the mixture of butter fat and salts and fed whole. The last method was the least satisfactory, because some of the rats at times ate the embryos, leaving a part of the bran and endosperm uneaten, so that their diet was not strictly representative of the entire grain. As these foods were much lower in calorific value than our ordinary mixtures containing 25 to 30 per cent of fat, the rats ate about 50 per cent more of these rations than of our "standard" foods, and thus actually consumed as much total protein as rats on our ordinary fat-rich diets which contain 15 to 17 per cent protein. This is a further illustration of how misleading may be conclusions based on the *percentage* of protein in the food when nothing is known regarding the *amount* of food and protein *eaten*.[24]

These experiments, for which graphic records are shown in Chart II, demonstrate that the wheat proteins considered in their entirety are adequate for promoting normal growth if eaten in sufficient amount.

We can thus substantiate the evidence that the proteins of the entire wheat kernel suffice to promote the growth of rats to normal adult size. The quantity of protein required is, however, relatively large.

Wheat Embryo as a Source of Protein.

The embryo constitutes about 1.5 per cent of the entire kernel. The protein value of the embryo comes into prominence in milling by-products, although from a *protein* standpoint when the whole wheat is used the embryo can play a small rôle at best. Commercial wheat embryo meal contains relatively large amounts of both bran and endosperm, the proportions often being quite

[24] This point has been discussed elsewhere; see Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1918, xxxiv, 521.

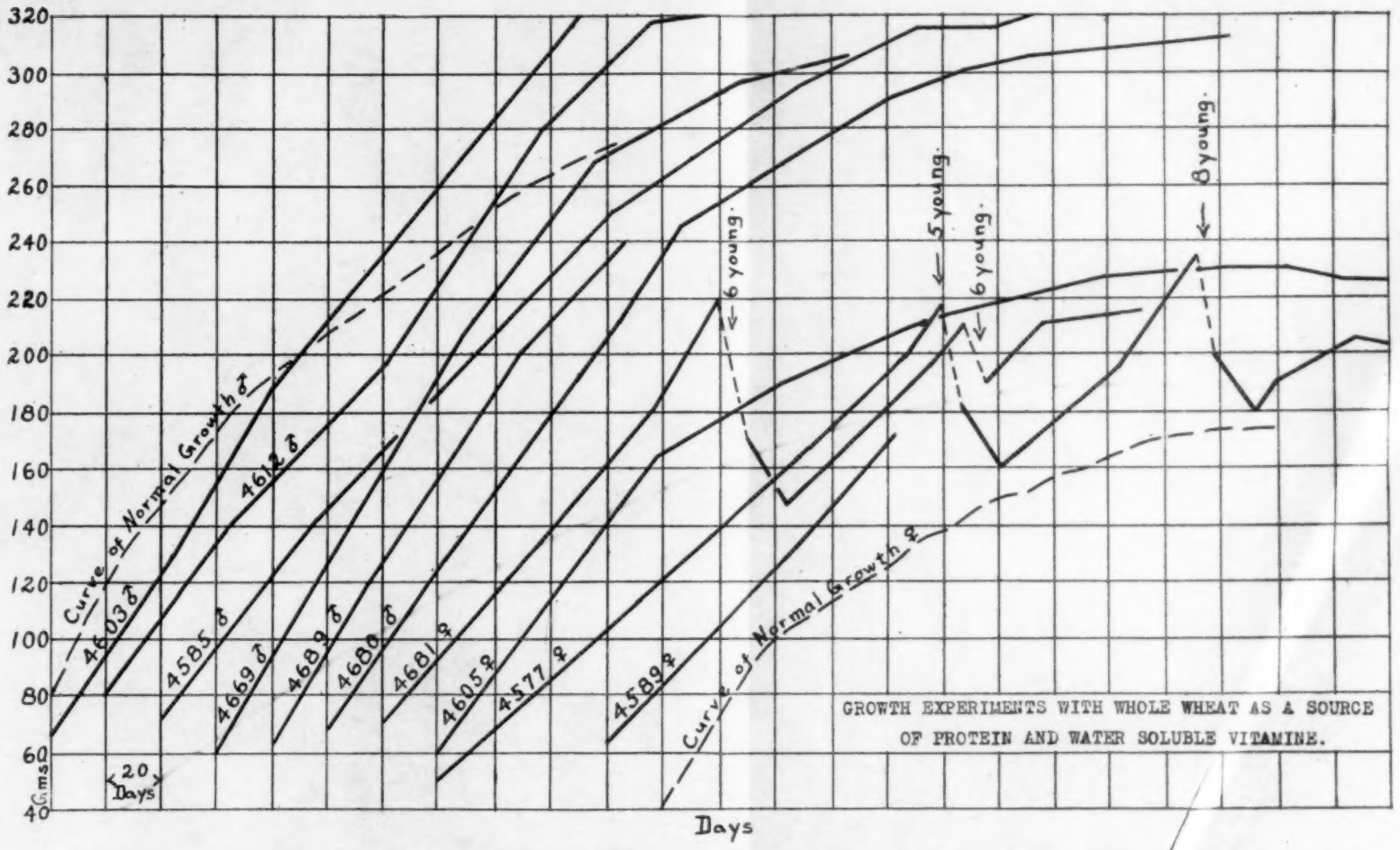
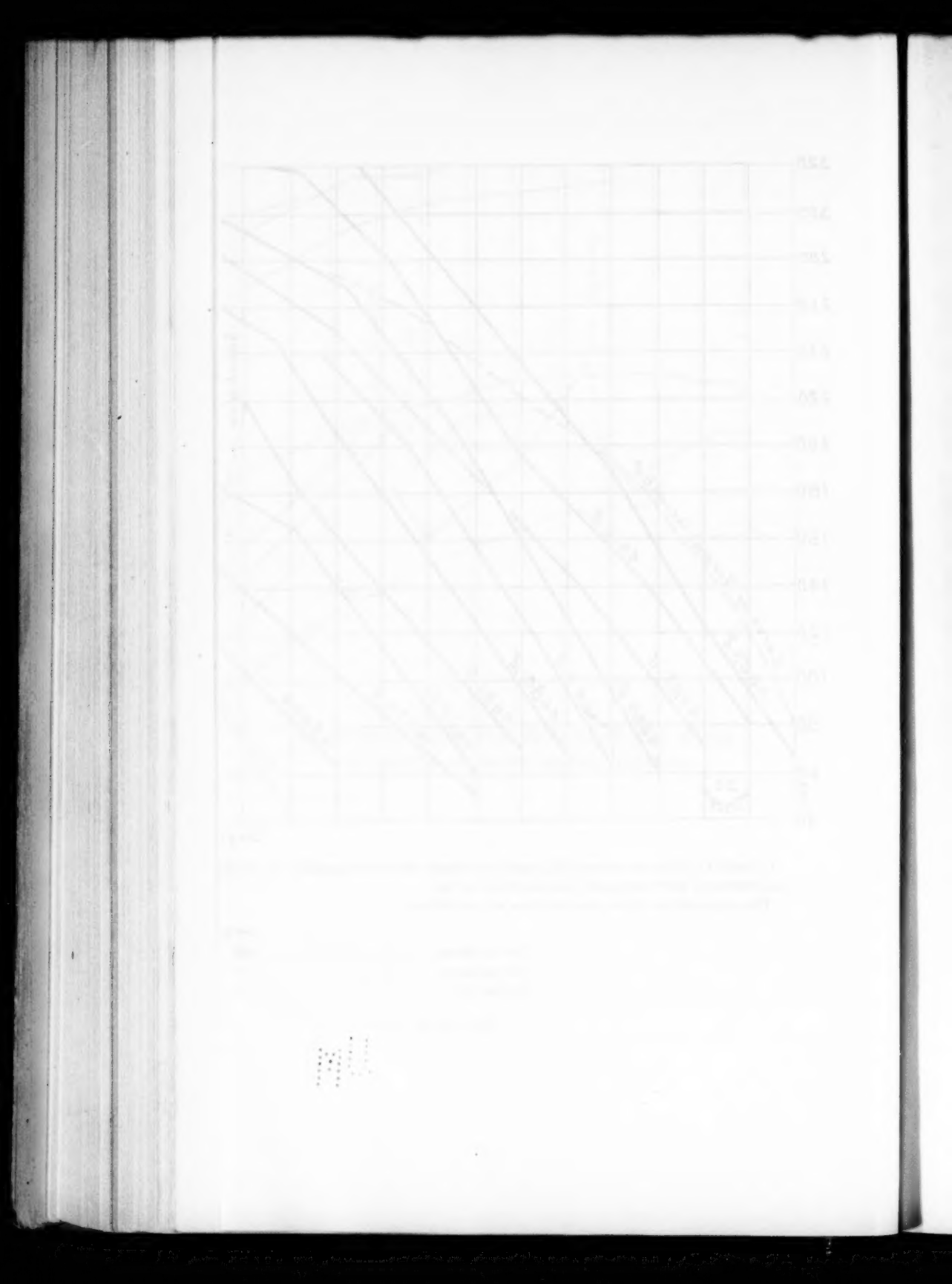


CHART II. Rats grow normally and reproduce on a diet in which all of the protein and water-soluble vitamine is furnished solely by *whole wheat*, supplemented with inorganic salts and butter fat.

The composition of the food mixture was as follows:

	per cent
Whole wheat	92 = 10 per cent protein (N × 5.7)
Salt mixture*	3
Butter fat	5

* See legend, Chart I.



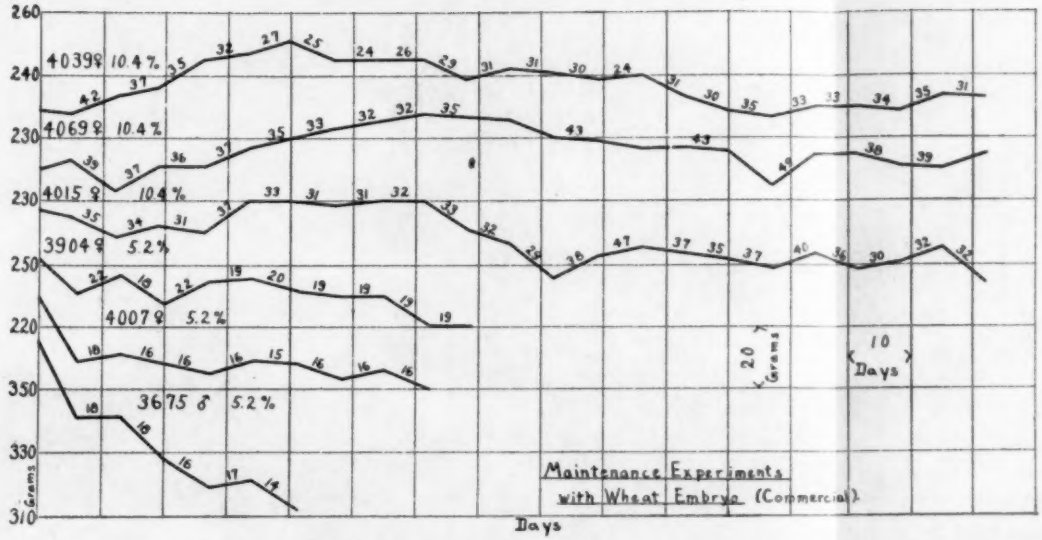


CHART III. The figures on the curves show the weekly intake of protein per gm. of body weight. Rats are maintained on diets in which the protein and water-soluble vitamine are furnished solely by *commercial wheat embryo*, supplemented with inorganic salts and butter fat.

Rats 3904 and 4007 were maintained for some weeks on a protein intake of less than 20 mg. per gm. of body weight. Rat 3675 on a protein intake as great as that of Rat 4007 however declined.

The composition of the food mixtures was as follows:

	5.2 per cent protein (N × 6.25).	10.4 per cent protein (N × 6.25).
	per cent	*per cent*
Wheat embryo	19	38
Salt mixture*	3	3
Starch	53	34
Butter fat	9	9
Lard	16	16

* See legend, Chart I.

unlike in different samples. The preparation used for these experiments, which contained about 50 per cent only of the pure embryo has already been described on page 561. Although preparations containing a larger proportion of embryo are obtainable, such were not available at the time these experiments were undertaken. From the results of our analyses of this preparation it appears that about 77 per cent of the nitrogen in the commercial embryo meal used for the experiments here described belongs to the pure embryo.

Maintenance Experiments.—Adult rats have been well maintained for several weeks on a diet containing about 10.4 per cent of "crude protein" (N × 6.25) derived entirely from commercial wheat embryo meal equivalent to about 8.1 per cent of "crude protein" from the pure embryo (see Chart III). The quantities of food which these animals ate gave them a weekly protein intake of 24 to 50 mg. per gm. of body weight. That these quantities are above the minimum for maintenance is shown in Chart III by Rat 3904 ♀ which was maintained for 5 weeks on an average of 20 mg., and by Rat 4007 ♀, maintained for 6 weeks on about 16 mg. of the commercial embryo protein per week, the "crude protein" content of their rations being 5.2 per cent. Both of these rats, however, declined slightly, during the period of feeding as a whole, and Rat 3675 ♂ declined rapidly, although the protein intake per gm. of body weight was quite as large as that of Rat 4007. Why this rat declined is not clear, especially as subsequently it recovered promptly on a mixed diet.

Our evidence is scarcely sufficient to justify final conclusions as to the minimum quantity of embryo protein required for maintenance. However, the data indicate that the "crude protein" in this commercial embryo meal was more efficient for maintenance than is that of the entire wheat kernel.

Growth Experiments.—The proteins of the wheat embryo are much more efficient than those of the entire kernel for promoting growth. Diets containing as little as 7.0 per cent of "crude protein" (N × 6.25) of which 77.1 per cent was from the embryo, 14.2 per cent from bran, and 8.7 per cent from endosperm, induced considerable growth in rats (Chart IV, Rats 4580 ♂, 4571 ♂, 4587 ♀, 4573 ♀, Period 1); and when the protein amounted to 13.7 per cent the animals made practically normal growth during

several months (Chart IV, Rats 4621 ♀, 4601 ♂, 4608 ♂, 4604 ♀, and Rats 4580 ♂, 4571 ♂, 4587 ♀, 4573 ♀, Period 2).

Table I shows the growth-promoting value of the proteins of the embryo of wheat in comparison with those of wheat bran,

TABLE I.

Comparisons of Gains of Body Weight per Gm. of Ingested Protein When Furnished by Wheat Embryo, Wheat Bran, Casein, or Lactalbumin.

Source of protein.	Protein in food.	Rat.	Initial body wt.	Gain in 4 wks.	Total intake.		Intake per gm. of gain.		Gain per gm. of protein.
					Food.	Protein.	Food.	Protein.	
	per cent		*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
Wheat embryo	13.7	4621 ♀	60	41	208	28.5	5.1	0.69	1.44
		4608 ♂	74	52	232	31.8	4.5	0.61	1.63
		4604 ♀	59	42	204	28.0	4.9	0.67	1.50
		4601 ♂	59	56	225	30.8	4.0	0.55	1.82
Average							4.6	0.63	**1.60**
Wheat embryo	6.95	4571 ♂	64	21	206	14.3	9.8	0.68	1.47
		4573 ♀	67	25	220	15.3	8.8	0.61	1.63
		4580 ♂	62	30	243	16.9	8.1	0.56	1.78
		4587 ♀	70	31	282	19.6	9.1	0.63	1.59
Average							9.0	0.62	**1.61**
Wheat bran	9.05	4635 ♂	67	44	280	21.5*	6.4	0.49*	**2.05**
Casein	11.0	2117 ♂	69	38	159?	17.5?	4.2	0.46	2.17
		2623 ♂	65	62	217	23.8	3.5	0.38	2.60
		2630 ♂	63	67	233	25.6	3.5	0.38	2.61
Average							3.6	0.41	**2.46**
Lactalbumin	10.3	2472 ♂	62	37	166	17.1	4.5	0.46	2.16
		2474 ♂	71	60	213	21.9	3.6	0.37	2.74
		2931 ♂	63	47	189	19.5	4.0	0.42	2.41
Average							4.0	0.41	**2.44**

* 15 per cent deducted on account of poor utilization of this food.

milk casein, or lactalbumin, established according to the method recently developed in this laboratory.[25] The average amount

[25] Osborne, T. B., Mendel, L. B., and Ferry, E. L., *J. Biol. Chem.*, 1919, xxxvii, 223.

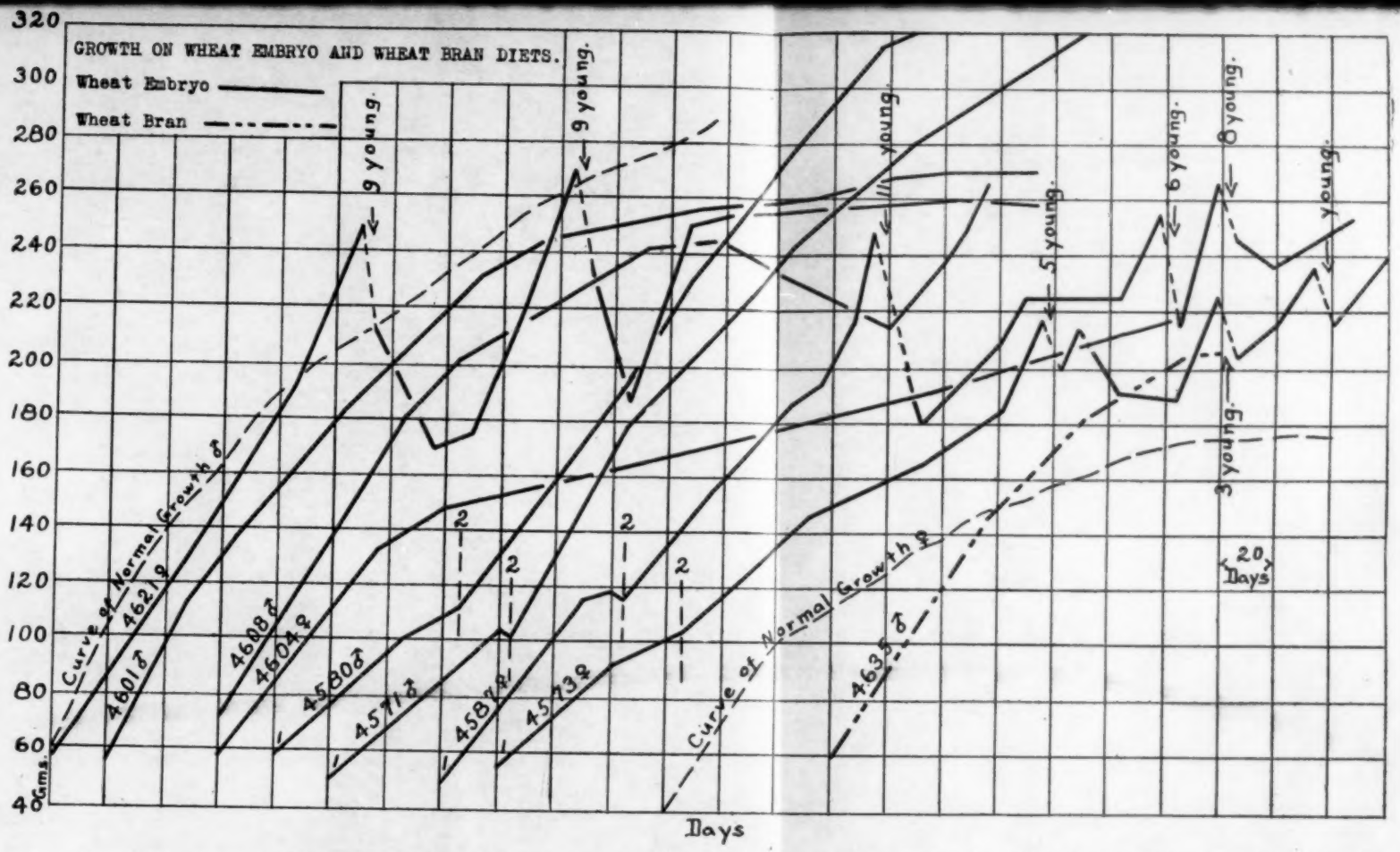


CHART IV. When protein and water-soluble vitamine were furnished solely by *commercial wheat embryo* fairly good growth was made by rats when the diet contained only 6.9 per cent of protein (Rats 4580, 4571, 4587, 4573, Period 1). Much better growth was made when the ration contained 13.7 per cent of protein (Rats 4580, 4571, 4587, 4573, Period 2, and also Rats 4621, 4601, 4608, 4604). Several litters of young were produced on this diet. Although some of these young died, those that survived have grown at a practically normal rate (see Chart XIV).

Rat 4635 was on a diet in which the protein and water-soluble vitamine were furnished by 55 per cent of *commercial wheat bran*. The significance of this experiment in relation to the value of wheat bran proteins is discussed in the text.

The composition of the food mixtures was as follows:

	Rats 4621, 4601, 4608, 4604, and Rats 4580, 4587, 4571, 4573, Period 2.	Rats 4580, 4587, 4571, 4573, Period 1.	Rat 4635
	per cent	*per cent*	*per cent*
Wheat embryo	50	25.4	
" bran			55–58
Salt mixture*	3	3.0	2
Starch	22	46.6	18–12
Butter fat	9	9.0	9
Lard	16	16.0	16–19

* See legend, Chart I.

of gain per gm. of protein eaten during 4 weeks was 1.60 gm. In these experiments growth was obviously determined by the amount of protein eaten; hence this figure may be accepted as a fair numerical expression of the value of the embryo proteins for growth, and shows that the "crude protein" of the embryo is more efficient than that of the entire kernel and decidedly more so than that of the endosperm or wheat flour (see Tables II and III).

Wheat Bran as a Source of Protein.

Growth Experiments.—On a diet containing 55 per cent of commercial wheat bran, which furnished 9 per cent of protein (N × 6.25), 88.5 per cent of which was from bran and 11.5 per cent from endosperm, one rat grew at a nearly normal rate for several weeks, although three other animals on the same diet grew very little. Analysis of the feces of these four rats showed that 75 to 80 per cent of the total food and 70 to 75 per cent of the nitrogen was utilized. The one rat which grew well ate very liberal quantities of the food, proving that the "crude protein" in bran is adequate for growth if enough is eaten (see Chart IV, Rat 4635).

The data given in Table I show that the "crude protein" of wheat bran has a higher value for the growing animal than does that of the embryo. This one rat, which grew well on a diet containing 9.05 per cent of "crude protein" (N × 6.25) from bran gained 2.05 gm. in body weight per gm. of "crude protein" eaten. The "crude protein" of bran appears to be quite as efficient as that of the combination of wheat flour with egg, milk, or meat, under the conditions of this experiment (see Table II).

If we assume, as is almost certainly the case, that the proportion of *pure* protein in bran is less than that of the "crude protein," as estimated by multiplying the nitrogen by the conventional factor 6.25, the efficiency of bran protein, if it could be isolated and tested in the pure state, would unquestionably be found higher than that of the "crude protein." It must not be forgotten that our estimate of the value of the latter is based on a single experiment, and that we have offered no evidence to

prove that the maximum efficiency of the "crude bran protein" has been finally established. The concentration of protein in the bran diet fed, however, was so low that probably growth which was not quite normal, was limited by the amount of protein ingested. These data indicate that the agriculturist is justified in his high estimate of the value of wheat bran as a protein concentrate.

Wheat Endosperm as a Source of Protein.

For this purpose wheat flour (Famous XXX brand) containing, air-dry, 1.43 per cent N equal to 8.15 per cent protein (N × 5.7) was used. Efforts to obtain flour of higher protein content failed owing to the restrictions imposed on milling which prevailed at the time these experiments were begun. Since this flour was so low in protein, it was necessary, in most of the experiments, to increase the protein by adding wheat gluten, made by washing the starch out of the same lot of flour. The results obtained were presumably not seriously affected by this procedure because upwards of 80 per cent of the nitrogen of the endosperm was recovered in the gluten used.

Maintenance Experiments.—On diets containing about 7.5 per cent of protein, two-thirds from the wheat flour and one-third from gluten, all but one of these adult rats were very nearly maintained for many weeks (see Chart V). These animals ate on the average 23 to 26 mg. of protein per gm. of body weight per week, quantities essentially like those found to be adequate for maintenance on the entire kernel. One rat, No. 3932 ♂, steadily declined in weight on this intake until the protein of the gluten was replaced by an equivalent amount of meat residue, the total food intake being unchanged. 4 weeks later this rat was killed and found to have infected lungs. Whether or not this disease was the cause of its higher protein requirement cannot, of course, be determined. The addition of 0.1 gm. of wheat embryo daily (Rats 3983 ♀, 3953 ♀) caused the animals to be better maintained although their food intake was restricted to the quantity eaten during the previous period and their weekly protein intake, about 25 mg. per gm. of body weight, was practically unchanged. When the gluten was omitted and the endosperm diets contained only about 5 per cent of protein, giving the animals an average

weekly protein intake of about 16 mg. per gm. of body weight, all declined steadily, showing that for the protein of the wheat endosperm this quantity is too low. The *total* food intake during the period of decline had previously been found to be adequate for maintenance. When 1 per cent of protein as meat residue replaced an equal quantity of wheat flour proteins in this 5 per cent protein diet of the rats which were declining, and a small amount of commercial wheat embryo was fed, to compensate for the diminution of water-soluble vitamine due to replacing 20 per cent of the flour in the food with starch, the animals were maintained better, although their food intake was kept unchanged. Their weekly protein intake, including the small amount furnished by the wheat embryo, averaged about 18 mg. per gm. of body weight. These experiments, together with that on Rat 3932 in the period during which it had meat protein but no added embryo, indicate that when a suitable protein is used to supplement the amino-acid deficiencies of the wheat proteins *less* protein is required for maintenance than when wheat flour is the sole source of protein.

Growth Experiments.—Young rats have made almost no growth on diets all the protein of which was furnished by wheat flour, in spite of the fact that they consumed as much food, as well as protein per week, as the majority of rats of comparable size which have grown well on our ordinary rations, similar in calorific value and protein content. When fed on these wheat flour diets rats whose final average body weight was 81 gm. ate on the average 37 gm. per week, whereas the majority of normally growing animals of 80 gm. of body weight eat weekly about 38 gm. of food of like calorific value.[26] Hence we can conclude that the failure of these rats to grow (see Chart VI, Series A) was not due to insufficient food intake. The failure to grow therefore may have been due to either a deficiency of vitamine, to poor quality of the flour proteins, or to both of these factors combined.

Experiments with this flour as the sole source of water-soluble vitamine in otherwise adequate diets have led us to conclude that even 60 per cent furnished less than the optimum amount of vitamine for growth (see p. 591). We accordingly increased the amount of vitamine supplied to another series of rats (Chart VI,

[26] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1915, xx, 351.

CHART V. The figures on the curves show that a weekly protein intake of 23 to 26 mg. per gm. of body weight barely suffices to maintain adult rats when both protein and water-soluble vitamine are furnished solely by *wheat endosperm* (flour). When the water-soluble vitamine was increased by feeding a little commercial wheat embryo separately and the quality of the protein improved by replacing one-fifth of the endosperm protein by meat protein, Rat 3979 was maintained on an average weekly protein intake of 16 mg. per gm. of body weight. The proteins of flour are more economically utilized in a mixed diet containing meat and supplying sufficient water-soluble vitamine. Rat 3932 shows the effect of improving the quality of the protein without changing the vitamine content of the ration. The failure of this rat to be maintained on a protein intake equal to that of Rats 3983, 3953, 3974, and 3973 may possibly be due to higher vitamine requirement owing to its relatively large size. That this may be so is indicated by other experiments which we are now making.

The composition of the food mixtures was as follows:

	5 per cent protein (N × 5.7).		7.5 per cent protein (N × 5.7).	
	Period 1. Rats 3979, 3972, 3971, 3942, 3947	Period 2. Rats 3979, 3972, 3971	Period 1. Rats 3983, 3953, 3974, 3973, 3932	Period 2. Rat 3932 Period 3. Rats 3979, 3972, 3971
	per cent	*per cent*	*per cent*	*per cent*
Wheat flour	62.0	50.0	62.0	60.0
" gluten			3.3	
Meat residue*		1.2		3.0
Salt mixture†	3.0	4.0	3.0	3.0
Starch	10.0	19.8	6.7	9.0
Butter fat	9.0	9.0	9.0	9.0
Lard	16.0	16.0	16.0	16.0

* See Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1917, xxxii, 313.

† See legend, Chart I.

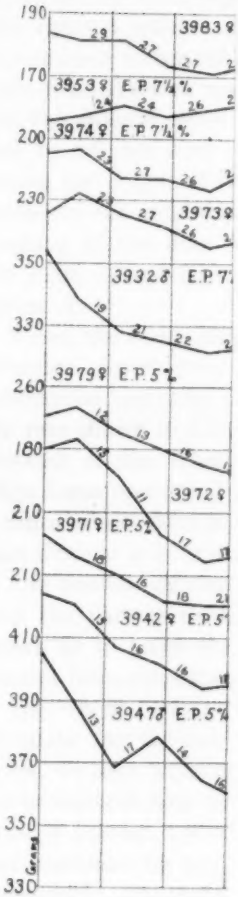

190
170
200
230
350
330
260
180
210
210
410
390
370
350
330
Grams
3983♀
3953♀ E.P. 7½%
3974♀ E.P. 7½%
3973♀
3932♂ E.P. 7
3979♀ E.P. 5%
3972♀
3971♀ E.P. 5%
3942♀ E.P. 5%
3947♂ E.P. 5%

CHART V. The figures on the curves show that a weekly protein intake of 23 to 26 mg. per gm. of body weight barely suffices to maintain adult rats when both protein and water-soluble vitamine are furnished solely by *wheat endosperm* (flour). When the water-soluble vitamine was increased by feeding a little commercial wheat embryo separately and the quality of the protein improved by replacing one-fifth of the endosperm protein by meat protein, Rat 3979 was maintained on an average weekly protein intake of 16 mg. per gm. of body weight. The proteins of flour are more economically utilized in a mixed diet containing meat and supplying sufficient water-soluble vitamine. Rat 3932 shows the effect of improving the quality of the protein without changing the vitamine content of the ration. The failure of this rat to be maintained on a protein intake equal to that of Rats 3983, 3953, 3974, and 3973 may possibly be due to higher vitamine requirement owing to its relatively large size. That this may be so is indicated by other experiments which we are now making.

The composition of the food mixtures was as follows:

	5 per cent protein (N × 5.7).		7.5 per cent protein (N × 5.7).	
	Period 1. Rats 3979, 3972, 3971, 3942, 3947	Period 2. Rats 3979, 3972, 3971	Period 1. Rats 3983, 3953, 3974, 3973, 3932	Period 2. Rat 3932 Period 3. Rats 3979, 3972, 3971
	per cent	*per cent*	*per cent*	*per cent*
Wheat flour	62.0	50.0	62.0	60.0
" gluten			3.3	
Meat residue*		1.2		3.0
Salt mixture†	3.0	4.0	3.0	3.0
Starch	10.0	19.8	6.7	9.0
Butter fat	9.0	9.0	9.0	9.0
Lard	16.0	16.0	16.0	16.0

* See Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1917, xxxii, 313.

† See legend, Chart I.

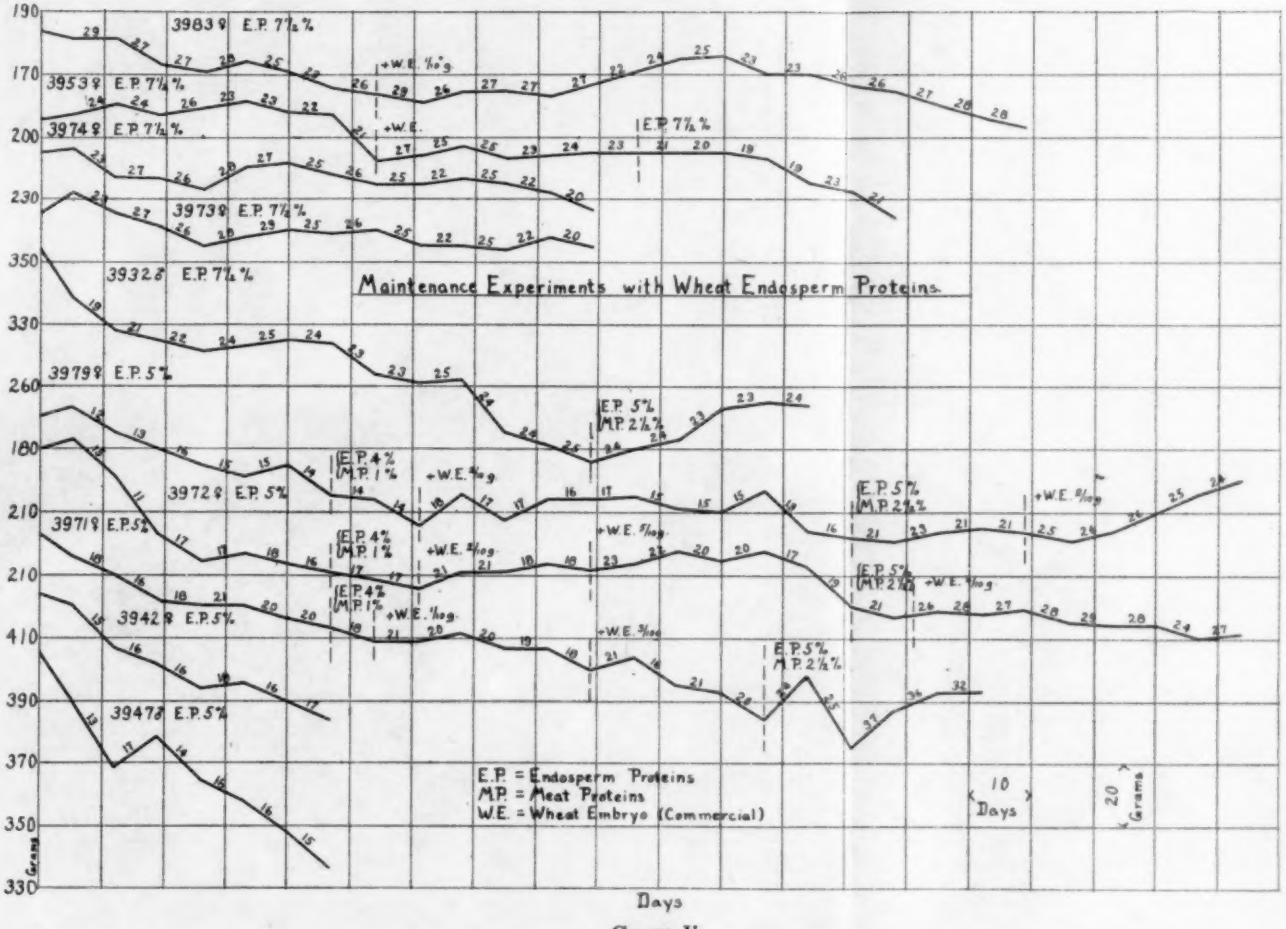
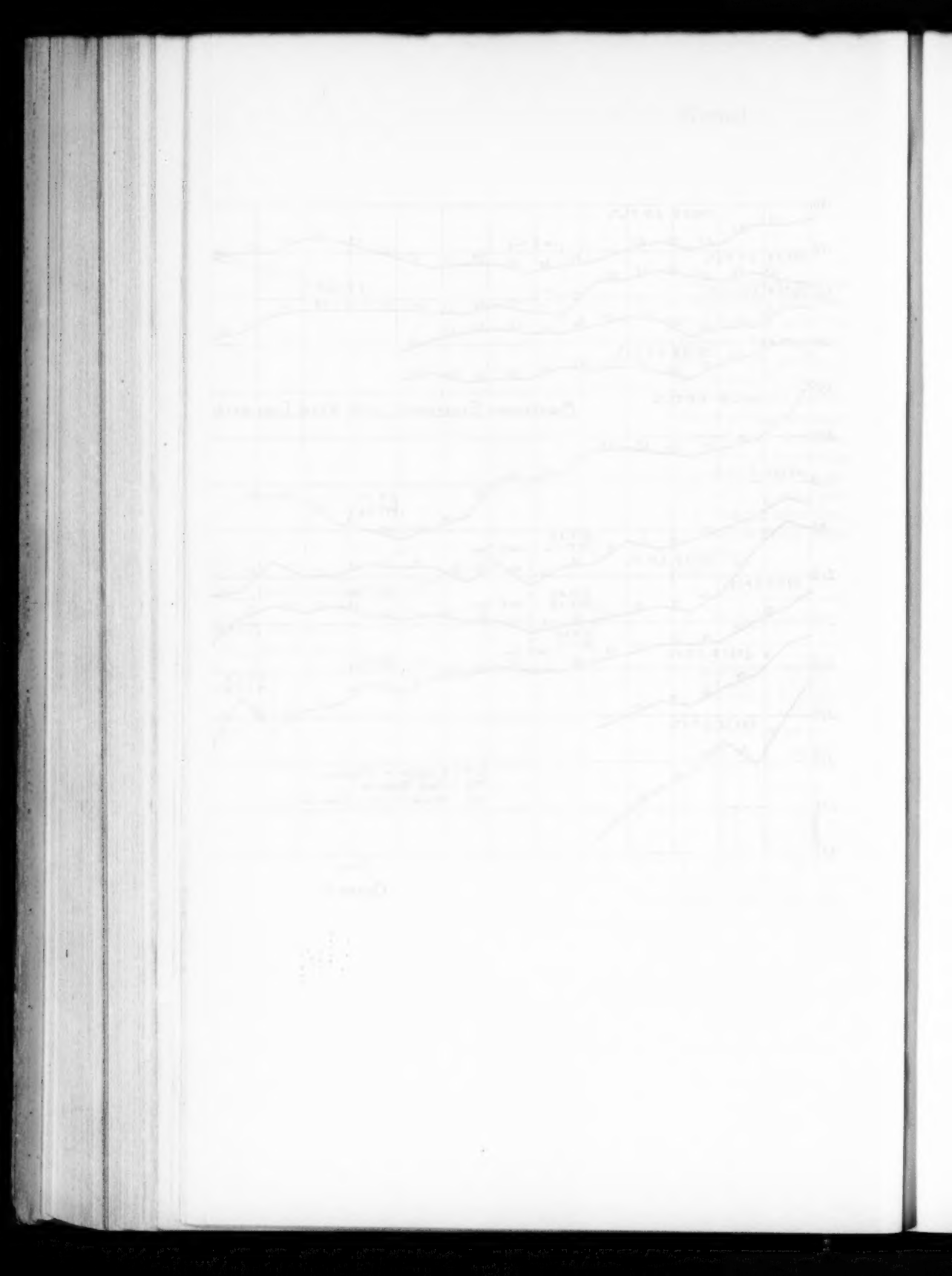

Maintenance Experiments with Wheat Endosperm Proteins.
3983♀ E.P. 7½%
3953♀ E.P. 7½%
3974♀ E.P. 7½%
3973♀ E.P. 7½%
3932♂ E.P. 7½%
3979♀ E.P. 5%
3972♀ E.P. 5%
3971♀ E.P. 5%
3942♀ E.P. 5%
3947♂ E.P. 5%
+W.E. ½ g.
+W.E.
E.P. 7½%
E.P. 5%
M.P. 2½%
E.P. 4%
M.P. 1%
+W.E. ¾ g.
+W.E. 1 g.
+W.E. 2 g.
+W.E. 3 g.
+W.E. 5 g.
E.P. = Endosperm Proteins
M.P. = Meat Proteins
W.E. = Wheat Embryo (Commercial)
10 Days
20 Grams
Grams
Days

CHART V.



Series B) which were otherwise fed on the same dietary as Series A, by giving them separately as much of the solids of a protein-free aqueous extract of dried yeast as had been proved by other experiments to furnish enough vitamine for nearly normal growth. This yeast fraction was equal to 16 per cent of the original yeast and the amount eaten contained nitrogen equal to protein equivalent to 1.6 per cent of the average amount of protein ingested during the course of these experiments. In spite of this large increase in the vitamine consumed and also of a possible supplementing of the flour protein by the nitrogenous constituents of the yeast extract these rats grew little if any better than those of Series A.

When the food contains adequate protein, but is deficient in vitamine, satisfactory growth always follows an addition of a substantial quantity of vitamine; hence we must conclude that the rats shown in Chart VI, Series A, failed to grow because the proteins of the wheat endosperm flour were inadequate, even when these amounted to 15 per cent of the diet.

Since wheat flour is rarely the sole source of protein in the human dietary it is quite as important to know the nutritive value of the protein mixtures resulting from the combination of flour with the various food products which are commonly eaten with bread, as to know that of the proteins of bread alone. Having learned from the experiments already described that the proteins of the wheat endosperm are adequate for maintenance, but inadequate for normal growth, even when liberal quantities are eaten, we next attempted to compare the rate of growth made on the proteins of flour alone with that made on corresponding quantities of protein, two-thirds of which was supplied by wheat flour and one-third by egg, milk, or meat, together with vitamine in presumably adequate quantity. Egg powder and whole milk powder (Merrell Soule) were used to furnish the proteins from egg and milk. The meat was furnished by lean round of beef which was dried and ground to a powder in the laboratory.[27] It was considered, in view of our other numerous experiments, that the quantity of these animal products, in conjunction with the 55 to 60 per cent of wheat flour included in these diets, would fur-

[27] Cf. Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1917, xxxii, 313.

nish an adequate amount of the water-soluble vitamine. Sufficient fat-soluble vitamine was provided for by liberal additions of butter fat.

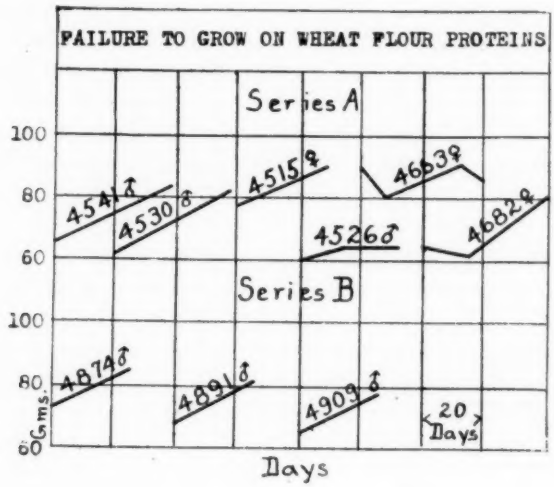


CHART VI. When wheat *endosperm* (flour + wheat gluten) furnishes the sole source of protein rats fail to grow (Series A). Scarcely any improvement results when the vitamine content of the food is increased by addition of a water extract of yeast (Series B).

The composition of the food mixtures was as follows:

	Series A.	Series B.
	per cent	*per cent*
Wheat flour	58	58
" gluten	13	13
Salt mixture.*	4	4
Butter fat	9	9
Lard	16	16
Yeast extract		0.2 gm. per day

* See legend, Chart I.

The results obtained with these diets, each of which contained nitrogen equal to 14.8 per cent of protein, are shown in Chart VII, Periods 1 and 4. When the supplementary proteins were re-

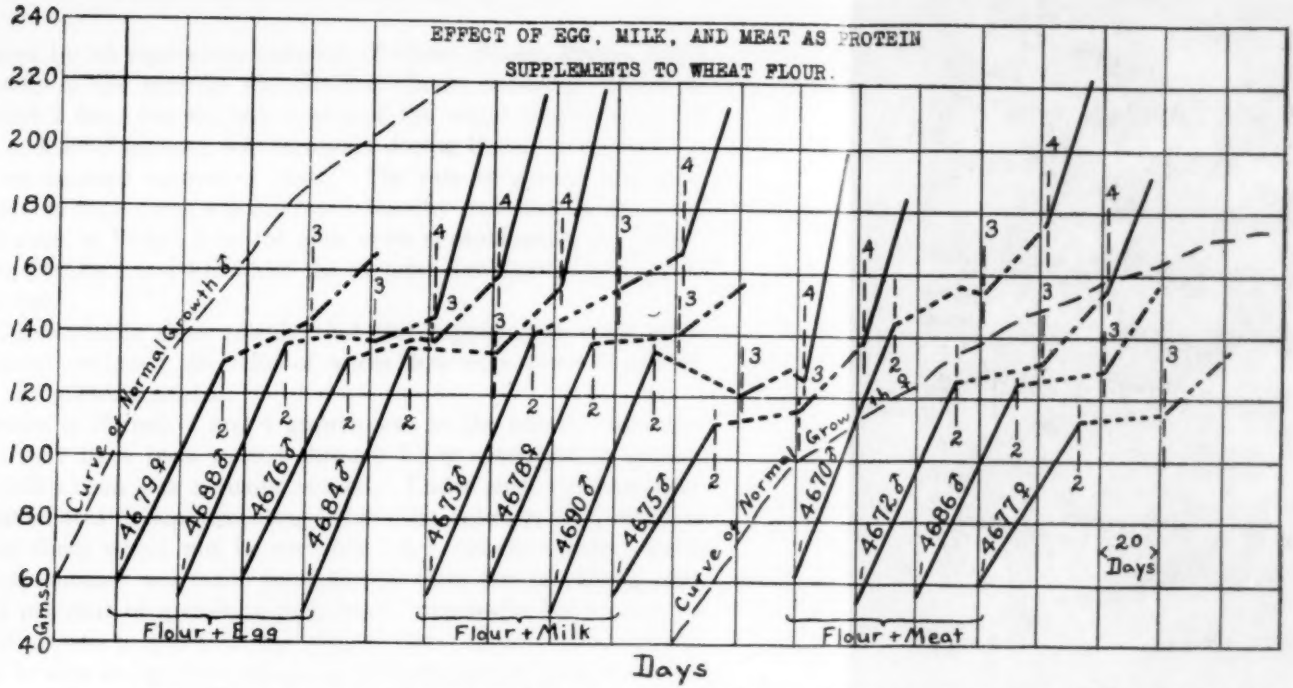


CHART VII. When egg, milk, or meat powders were combined with wheat flour in such proportion that one-third of the total protein of the ration was furnished by the animal product, Periods 1 and 4, growth was very much more rapid than when all of the protein was derived from flour alone, Period 2, or from flour and a small amount of yeast extract to supply more water-soluble vitamine, Period 3. Compare also Chart VI. Even though in Period 3 the yeast extract may have supplemented the flour proteins, and the food intake was greater than in Period 2, growth was very much slower than in Periods 1 and 4.

The composition of the food mixtures was as follows:

	Periods 1 and 4.			Periods 2 and 3. All rats.
	Rats 4679, 4688, 4676, 4684	Rats 4673, 4678, 4690, 4675	Rats 4670, 4672, 4686, 4677	
	per cent	*per cent*	*per cent*	*per cent*
Wheat flour	55.5	55.5	60.0	55.5
" gluten	8.1	8.1	6.6	15.5
Egg powder	11.5			
Milk "		20.0		
Meat "			5.9	
Salt mixture*	2.0	2.0	2.5	4.0
Butter fat	9.0	4.0	9.0	9.0
Lard	16.0	10.4	16.0	16.0

* See legend, Chart I.

placed by an equivalent quantity of wheat gluten, Period 2, the growth of the animals was checked almost instantly. Since in Period 2 flour was the sole source of the water-soluble vitamine an additional amount was furnished during Period 3, in the form of an aqueous extract of yeast. The rate of growth and food intake in most cases was increased thereby over that in Period 2, but even in Period 3 no rat grew even approximately as rapidly as in Periods 1 and 4 in which the superior combination of proteins was fed.

These results show plainly that the supplements—egg, milk, or meat—enhance the value of wheat flour as a source of protein for the growing animal.

Since in Periods 1 and 4 growth was at the normal rate, protein may have been wasted through being consumed in greater quantity than was actually needed. Under such circumstances if differences in supplementing value exist between eggs, milk, or meat these would not be revealed. Accordingly another series of experiments was made using similar diets but containing only 10.3 per cent of protein, a proportion supposedly low enough to limit growth to less than the normal rate. Inasmuch as growth was so slow on the diet containing 14.8 per cent of protein derived wholly from the wheat flour it seems fair to assume that on this diet growth was at the maximum rate possible with this proportion of protein.

When egg or milk powders were used to supplement the flour proteins no other source of water-soluble vitamine was deemed necessary. Since flour and meat powder are low in vitamine two series of experiments were made with the meat supplement, one with, the other without, extra vitamine supplied by 0.2 gm. of dried yeast fed separately each day. The fat-soluble vitamine was supplied by butter fat, allowance being made for the fat in the egg or milk powder, which was assumed to have a value equal to that of butter fat as a source of this factor. Additions of lard made the total amount of fat in each food 25 per cent, consequently the total calorific value of the several diets was practically identical. The results of these experiments are shown in Table II, from which it is seen that decidedly more rapid growth occurred when the diet contained only 10.3 per cent of protein, furnished by flour supplemented with egg, milk, or meat, than when it con-

tained even 50 per cent more protein derived entirely from the wheat flour.

The following summary shows the average relative gains of body weight, per gm. of protein eaten, made by the rats fed on these different diets.

	Per cent of protein in food.	Gain of body weight per gm. of protein.
		gm.
Flour + egg	14.8	2.00
	10.3	1.80
" + milk	14.8	1.67
	10.3	1.73
" + meat	14.8	1.73
	10.3	1.47
" + " + yeast	10.3	1.66
" + gluten	14.8	0.50

For the data from which these figures were derived see Table II. From them it appears that eggs or milk supplement flour proteins slightly more efficiently than does meat even though additional vitamine was supplied by yeast.

The fact that in the experiments with 10.3 per cent of protein the total gain of weight was so much less than with 14.8 per cent shows that in the former case protein was the limiting factor. Since the gains per gm. of protein eaten were so nearly alike for the two concentrations of protein, we are justified in the assumption that the higher figures represent approximately the maximum efficiency of these protein mixtures for promoting growth. We realize that such a mathematical expression of the relative merits of these food mixtures is not strictly justifiable, because if growth on the flour proteins alone had been sufficiently slow its amount, stated in gm., would approach zero, at which point the difference between the amounts of protein required would equal infinity when flour protein alone is contrasted with its combinations with the animal proteins.

Having thus obtained evidence of the effect on the nutritive

TABLE II.

(See Chart VII.)

Gain of Body Weight Per Gm. of Ingested Protein Furnished by Wheat Endosperm (Flour) Combined with Egg, Milk, or Meat, and by Endosperm Alone.

Source of protein.	Protein in food.	Rat.	Initial body wt.	Gain in 4 wks.	Total intake.		Intake per gm. of gain.		Gain per gm. of protein.
					Food.	Protein.	Food.	Protein.	
	per cent		gm.	gm.	calories*	gm.	calories*	gm.	gm.
Wheat flour " gluten Egg powder	14.8	4676 ♂	69	62	1,260	33.8	20.4	0.55	1.83
		4684 ♂	54	82	1,335	35.9	16.3	0.44	2.28
		4688 ♂	60	76	1,335	35.9	17.6	0.47	2.12
		4679 ♀	69	61	1,240	33.4	20.4	0.55	1.83
Average							18.7	0.50	**2.02**
Wheat flour " gluten Egg powder	10.3	5102 ♂	67	49	1,410	25.0	28.8	0.51	1.96
		5115 ♂	70	49	1,570	27.9	32.0	0.57	1.76
		5111 ♂	72	43	1,430	25.4	33.2	0.59	1.69
		5104 ♂	65	40	1,270	22.6	31.8	0.57	1.77
Average							31.5	0.56	**1.80**
Wheat flour " gluten Milk powder	14.8	4673 ♂	70	70	1,240	38.7	17.7	0.55	1.81
		4675 ♂	62	49	1,050	32.8	21.4	0.67	1.49
		4690 ♂	56	78	1,200	37.4	15.4	0.48	2.08
		4678 ♀	75	61	1,335	41.6	21.8	0.68	1.47
Average							19.1	0.60	**1.71**
Wheat flour " gluten Milk powder	10.3	5120 ♂	71	54	1,645	29.8	30.4	0.55	1.81
		5121 ♂	71	44	1,410	25.4	32.1	0.58	1.73
		5128 ♂	70	40	1,345	24.3	33.6	0.61	1.65
		5191 ♂	72	62	1,430	25.8	23.1	0.42	2.40
Average							29.8	0.54	**1.90**
Wheat flour " gluten Meat powder	14.9	4670 ♂	75	69	1,260	38.2	18.2	0.55	1.81
		4672 ♂	65	60	1,070	32.5	17.8	0.52	1.85
		4686 ♂	68	56	1,180	35.8	21.0	0.64	1.56
		4677 ♀	65	47	940	28.6	20.0	0.61	1.64
Average							19.3	0.58	**1.72**

* Estimated (protein + carbohydrates × 4.1, fat × 9.3 calories).

TABLE II—*Concluded.*

Source of protein.	Protein in food.	Rat.	Initial body wt.	Gain in 4 wks.	Total intake.		Intake per gm. of gain.		Gain per gm. of protein.
					Food.	Protein.	Food.	Protein.	
	per cent		*gm.*	*gm.*	*calories**	*gm.*	*calories**	*gm.*	*gm.*
Wheat flour	10.3	5116♂	70	31	1,190	22.4	38.4	0.72	1.38
" gluten		5129♂	72	28	1,000	18.8	35.8	0.67	1.49
Meat powder		5117♀	66	38	1,310	24.5	34.5	0.64	1.55
Average							36.2	0.68	**1.47**
Wheat flour	10.3	5236♂	72	31	1,070	19.9	34.5	0.64	1.56
" gluten		5245♂	73	37	1,095	20.4	29.6	0.55	1.82
Meat powder + 0.1 gm. yeast daily		5246♂	75	33	1,220	22.6	37.0	0.68	1.46
		5249♂	76	42	1,270	23.4	30.2	0.56	1.80
Average							32.8	0.61	**1.66**
Wheat flour	14.7	4874♂	75	11	860	25.3	78.1	2.30	0.43
" gluten + 0.2 gm. yeast extract daily		4891♂	68	14	915	26.9	65.4	1.92	0.52
		4909♂	65	13	805	23.7	62.0	1.82	0.55
Average							68.5	2.01	**0.50**

* Estimated (protein + carbohydrates × 4.1, fat × 9.3 calories).

value of wheat flour caused by combining it with those animal products most generally eaten together with bread, it remains to be determined whether equally good results may not be secured by leaving in the flour those parts which are customarily separated from it by the miller and subsequently used to supplement the protein deficiencies of the rations of farm animals. In making experiments to decide this question a little yeast was added to the diet because our earlier experience showed that the endosperm, bran, and embryo, fed alone in the amounts used, supplied too little vitamine for normal growth. Although the proteins of the yeast undoubtedly supplement those of the wheat, nevertheless we believe the results of these experiments are comparable with one another, as well as with those obtained with foods containing a like percentage; namely, 10.3 per cent of protein derived from whole wheat or combinations of flour with eggs, milk, or meat.

TABLE III.

Effects of the Bran and Embryo of Wheat as Protein Supplements to Wheat Flour.

Source of protein.	Protein in food.	Rat.	Initial body wt.	Gain in 4 wks.	Total intake.		Intake per gm. of gain.		Gain per gm. of protein.
					Food.	Protein.	Food.	Protein.	
Series A									
	per cent		*gm.*	*gm.*	*calories**	*gm.*	*calories**	*gm.*	*gm.*
Wheat flour, gluten, bran, embryo + 0.1 gm. yeast daily	10.3	5127 ♂	70	40	1,310	28.5	32.8	0.71	1.40
		5123 ♂	74	41	1,350	29.4	33.0	0.72	1.40
		5125 ♂	75	35	1,320	28.6	37.8	0.82	1.23
		5126 ♂	70	30	1,220	26.5	40.7	0.88	1.13
Average							36.1	0.78	**1.29**
Series B.									
Wheat flour, gluten, bran, embryo + 0.1 gm. yeast daily	10.3	5109 ♂	71	50	1,495	31.6	29.9	0.63	1.58
		5113 ♂	70	35	1,255	26.8	35.8	0.77	1.31
		5106 ♂	69	43	1,395	29.6	32.4	0.69	1.45
		5100 ♂	72	32	1,355	28.8	42.3	0.90	1.11
Average							35.1	0.75	**1.36**
Series C.									
Whole wheat	10.3	4689 ♂	63	44	1,310	35.2	29.5	0.80	1.25
		4669 ♂	60	45	1,235	33.2	27.2	0.74	1.36
		4680 ♂	68	39	1,100	29.6	27.9	0.76	1.32
Average							28.2	0.77	**1.31**
Series D.									
Wheat flour, gluten + 0.2 gm. yeast	10.3	5086 ♂	68	27	1,345	27.0	49.8	1.00	1.00
		5083 ♂	71	29	1,235	25.0	42.6	0.86	1.16
		5089 ♂	75	26	1,070	21.9	41.2	0.84	1.19
		5094 ♂	71	26	1,290	26.0	49.6	1.00	1.00
Average							45.8	0.93	**1.09**

* Estimated (protein + carbohydrates × 4.1, fat × 9.3 calories).

In order to compare the supplementing effect of bran and embryo protein on the growth-promoting power of the endosperm protein with that of the protein of eggs, milk, or meat, a mixture of bran and embryo in the proportion in which these occur in the whole wheat kernel was used to furnish one-third of the protein; *i.e.*, the same proportion as did the animal products in the series just discussed. The results of these experiments are shown in Table III, Series A.

From the data given in Table III we calculate that 1 gm. of protein in the ration fed to Series A caused an average gain of body weight of 1.29 gm., somewhat more than when flour and yeast supplied the protein, Series D, but decidedly less than when the flour was supplemented by comparable quantities of the animal proteins (Table II).

To learn what supplementing effect the bran and embryo proteins have on the flour proteins when used in the same proportions as they exist in the entire wheat kernel, another series of rats was fed on a diet fulfilling these conditions (Table III, Series B).

The diets supplied to Series A and B were much alike and closely resembled in protein content that used for Series C which had 92 per cent of whole wheat mixed with 5 per cent of butter fat and 3 per cent of salts, but with no added yeast. It is interesting to note that growth was at approximately the same rate in each series; namely, 1.29, 1.36, and 1.31 gm. respectively per gm. of protein eaten. The conclusion therefore seems justified that in these experiments the yeast had no appreciable supplementing effect in Series A and B.

Wheat as a Source of Water-Soluble Vitamine.

Literature.—McCollum and Davis[28] found that a ration containing 64 per cent of whole wheat, supplemented by casein and suitable inorganic salts, failed to promote continued growth and normal reproduction of rats unless butter fat was also added to the diet. From this they conclude that the wheat kernel contains little of the fat-soluble vitamine.

McCollum, Simmonds, and Pitz[17] state that as small an amount as 15 per cent of whole wheat furnishes enough water-soluble vita-

[28] McCollum, E. V., and Davis, M., *J. Biol. Chem.*, 1915, xxi, 615

mine for completion of growth of rats and the production of a nearly normal number of young, but not enough to enable the young to develop to the weaning age.

The same authors[18] found that a commercial preparation of wheat embryo contained a moderate amount of the fat-soluble and so large an amount of the water-soluble vitamine that 2 per cent in the diet was sufficient for growth at the normal rate for several months.

The fact that the results of experiments showing the presence of the water-soluble vitamine in food products agree so well with those showing the presence of the antineuritic principle has led to the belief that the symptoms following the absence of these dietary factors from the food are due to a deficiency in the ration of intimately associated, if not identical, substances. Evidence that the antineuritic dietary constituent is unequally distributed in different parts of the seed is afforded by the newly published paper of Voegtlin, Lake, and Myers[29] who reach the following conclusions:

"For pigeons an exclusive diet of whole wheat or corn furnishes an adequate supply of antineuritic vitamine. The antineuritic vitamine seems to reside in the peripheral layers and the germ of these seeds, whereas the endosperm is relatively poor in this substance. If wheat and corn foods containing only a small percentage of the peripheral layers and germ of the seed are fed to pigeons and chickens exclusive of other food, polyneuritic symptoms appear on an average of three weeks after the beginning of the feeding period. The appearance of polyneuritis is preceded by a gradual loss in body weight. The birds can be relieved of their paralysis in a striking way by the oral or subcutaneous administration of a highly concentrated preparation of antineuritic vitamine derived from 'whole wheat' bread, yeast, ox liver, rice polishings, or beans. The addition of yeast (in amounts used by bakers) in the preparation of bread from highly milled flour does not prevent the appearance of polyneuritis in birds fed on this food, but prolongs slightly the period of incubation. The addition to 'highly milled' flour, or bread made from 'highly milled' flour, of a small amount of antineuritic vitamine preparation will correct this particular dietary deficiency, and will prevent the appearance of polyneuritis and the loss of body weight."

Experimental.—Adult rats fed on an otherwise adequate diet, in which the water-soluble vitamine was supplied wholly by 20 per cent of *wheat flour* (see Chart VIII, Period 1) lost weight steadily

[29] Voegtlin, C., Lake, G. C., and Myers, C. N., *Public Health Rep.*, 1918, xxxiii, 647, wherein some of the earlier literature on this topic is cited.

and quite rapidly. When the flour was replaced in Period 2 by an equal amount of finely ground *whole wheat* they were fully maintained for a long time. When later (in Period 3) they were returned to the wheat flour diet all lost weight, but promptly recovered in Period 4 when they were given water-soluble vitamine in the form of commercial wheat embryo, whole wheat, or yeast.

Since the impaired condition, caused by a diet deficient in water-soluble vitamine, is always followed by a loss of appetite, and the improved condition resulting from adding more of this factor to the diet is always accompanied by a marked increase in food intake, it is very difficult to compare the relative vitamine content of such food products as wheat flour and whole wheat.

An illustration is afforded by Rat 3928, Chart VIII, which, in respect to its food intake, behaved like the others. During the 2nd, 3rd, and 4th weeks of Period 1 this rat ate as much of the 20 per cent flour food per week as it ate of the 20 per cent whole wheat food during any week of Period 2. However, during the last week of Period 1 its food intake fell to about two-thirds of that previously eaten. In Period 2 its food intake rose at once, so that it ate as much as at the beginning of Period 1. Its body weight increased correspondingly and remained constant when restricted during the rest of this period to the same amount of food as that eaten in the 1st week. In Period 3 its food intake during the 1st week was the same as that in Period 2, but during the two subsequent weeks fell rapidly, accompanied by a rapid loss of body weight. Of course with declining food intake on a food containing a fixed percentage of vitamine the quantity of this factor received by the animal decreases proportionately, hence the animal quickly goes from bad to worse. Under such circumstances it is evident that comparisons based on the amount of vitamine actually consumed cannot be made. We have consequently been compelled to make this comparison on a percentage basis which, for practical purposes, furnishes information of value to those who use these products.

Growth.—In agreement with the experience of McCollum, Simmonds, and Pitz[17] we have found, that provided they eat enough, *young rats* grow well when only 15 per cent of the *entire kernel* supplies the water-soluble vitamine in an otherwise adequate ration having an energy value of 5 calories per gm. This proportion

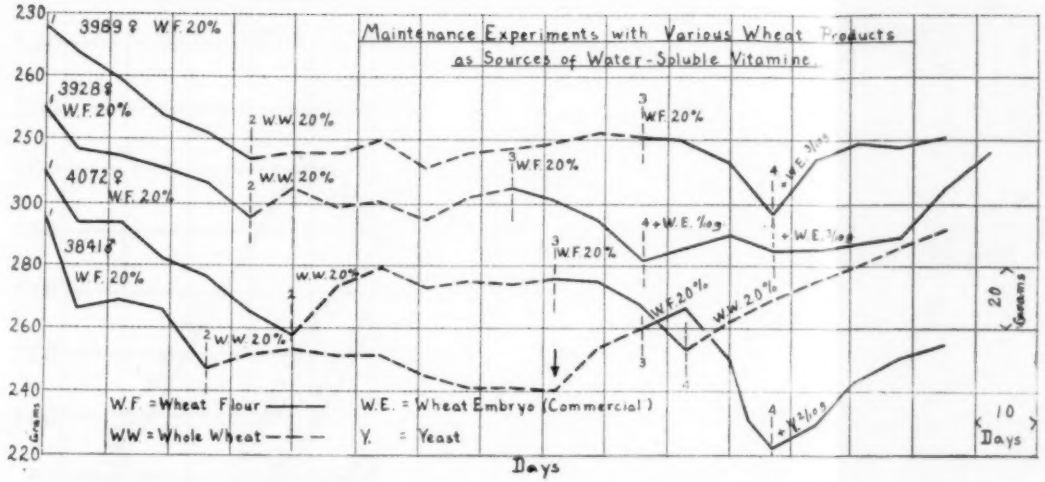


CHART VIII. *Wheat flour* (W.F.) is inferior to whole wheat (W.W.) as a source of water-soluble vitamine when either was supplied in the same proportions to the same animal in successive periods. The failures were not due to a lack of suitable protein, for casein was supplied in abundance. The decline in weight when wheat flour was used could promptly be averted by separately feeding small doses of commercial wheat embryo (W.E.) or yeast (Y). This always increased the food intake. Rat 3841 for which the food intake had been restricted in Period 2 to the quantity on which it had failed in Period 1, was fed *ad libitum* after the day indicated by the arrow.

The composition of the food mixtures was as follows:

	Whole wheat food.	Wheat flour food.
	per cent	*per cent*
Casein	15	15
Whole wheat	20	
Wheat flour		20
Salt mixture*	3	3
Starch	37	37
Butter fat	9	9
Lard	16	16

* See legend, Chart I.

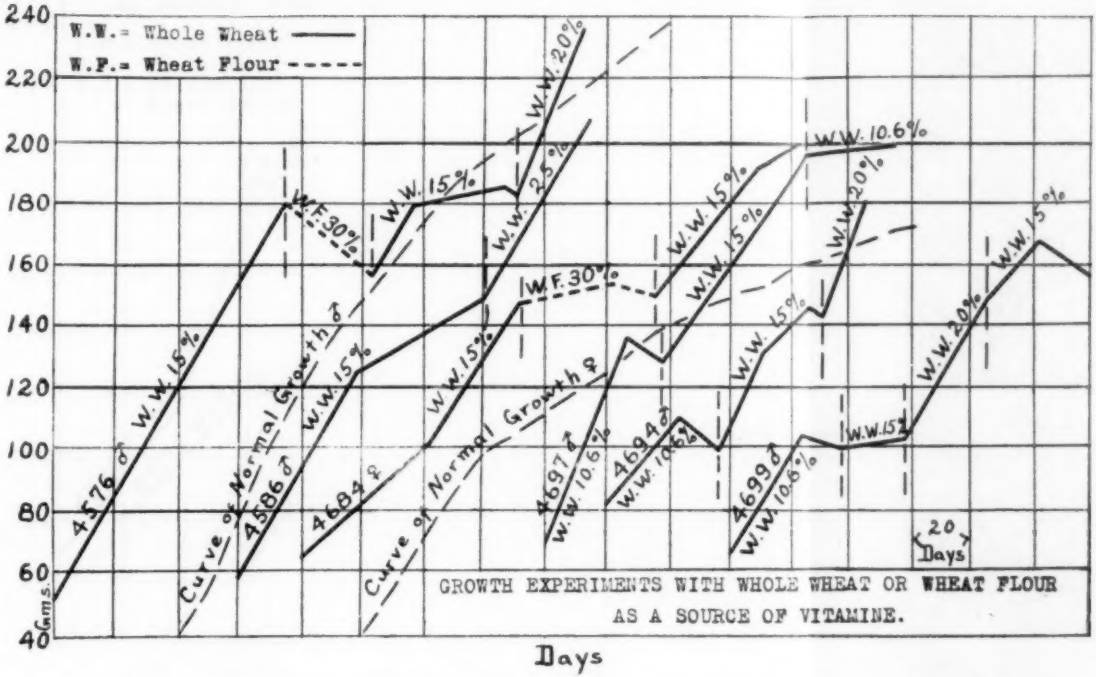
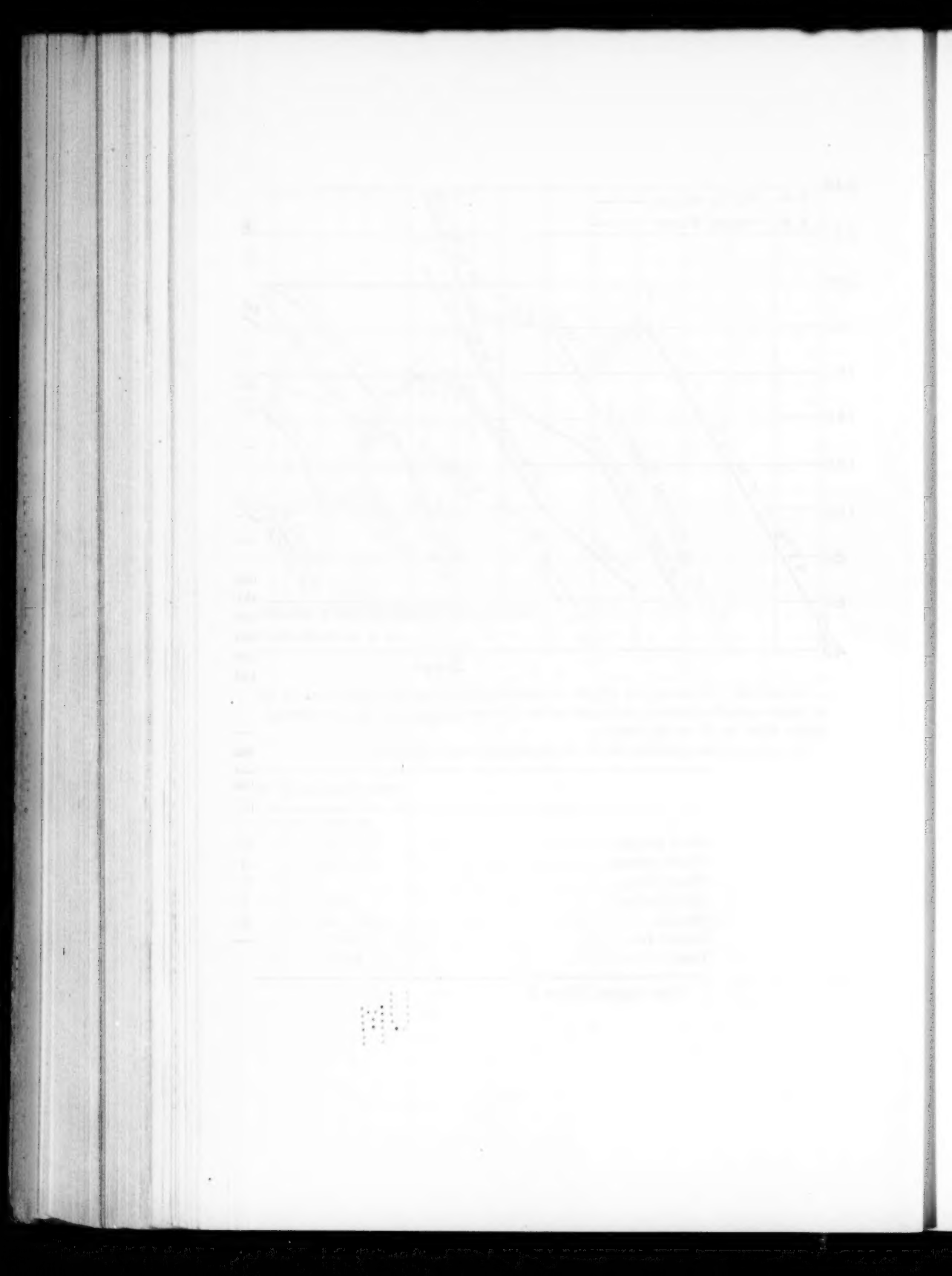


CHART IX. Showing the effects of additions of varying proportions of *whole wheat* (W.W.) as sources of *water-soluble vitamine* in an otherwise adequate ration. The percentage of total protein was essentially alike in all of the diets.

The general composition of the food mixtures was as follows:

	Whole wheat food.	Wheat flour food.
	per cent	*per cent*
Meat residue	15.0–16.7	15.5
Whole wheat	10.6–25.0	
Wheat flour		30.0
Salt mixture*	3.0	3.0
Starch	44.7–32.0	26.5
Butter fat	9.0	9.0
Lard	16.0	16.0

* See legend, Chart I.



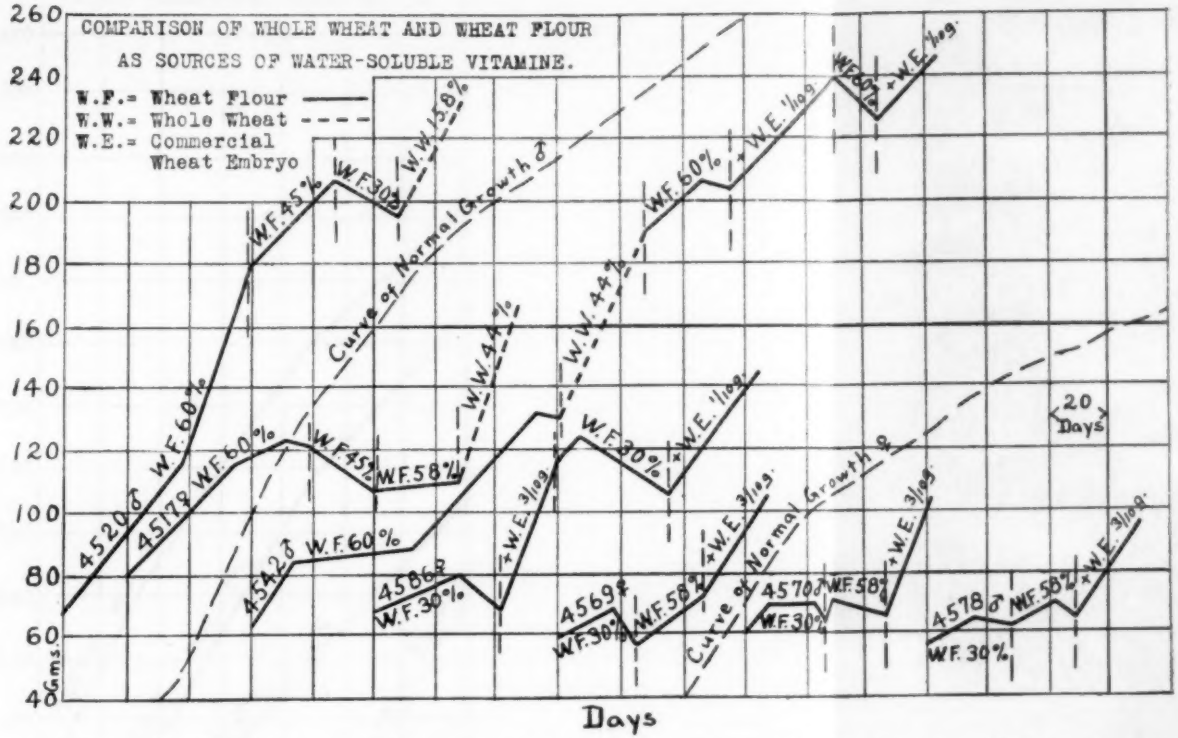
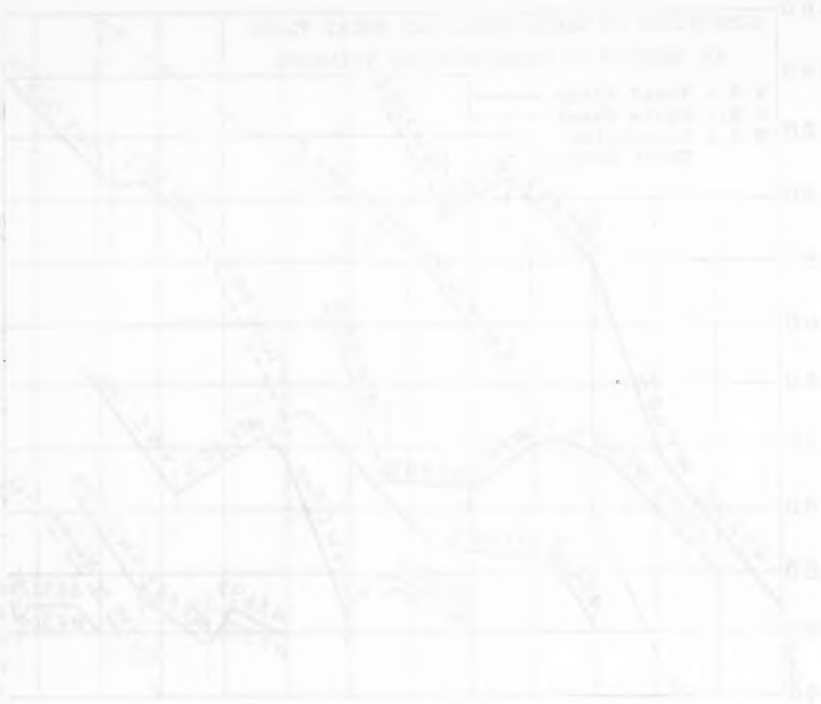


CHART X. Showing contrasts between the effects of different proportions of *whole wheat* (W.W.) and wheat flour (W.F.) as sources of *water-soluble vitamine* in diets otherwise adequate and comparable. The comparative efficiency of very small quantities of commercial wheat embryo (W.E.) is also shown.

The general composition of the food mixtures was as follows:

	Whole wheat food.	Wheat flour food.
	per cent	*per cent*
Meat residue*	11.5–16.0	11.5–15.5
Whole wheat	44.0–15.8	
Wheat flour		60.0–30.0
Salt mixture†	3.0	3.0– 4.0
Starch	16.5–40.2	0.0–26.5
Butter fat	9.0	9.0
Lard	16.0	16.0

* See Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1917, xxxii, 313.
† See legend, Chart I.



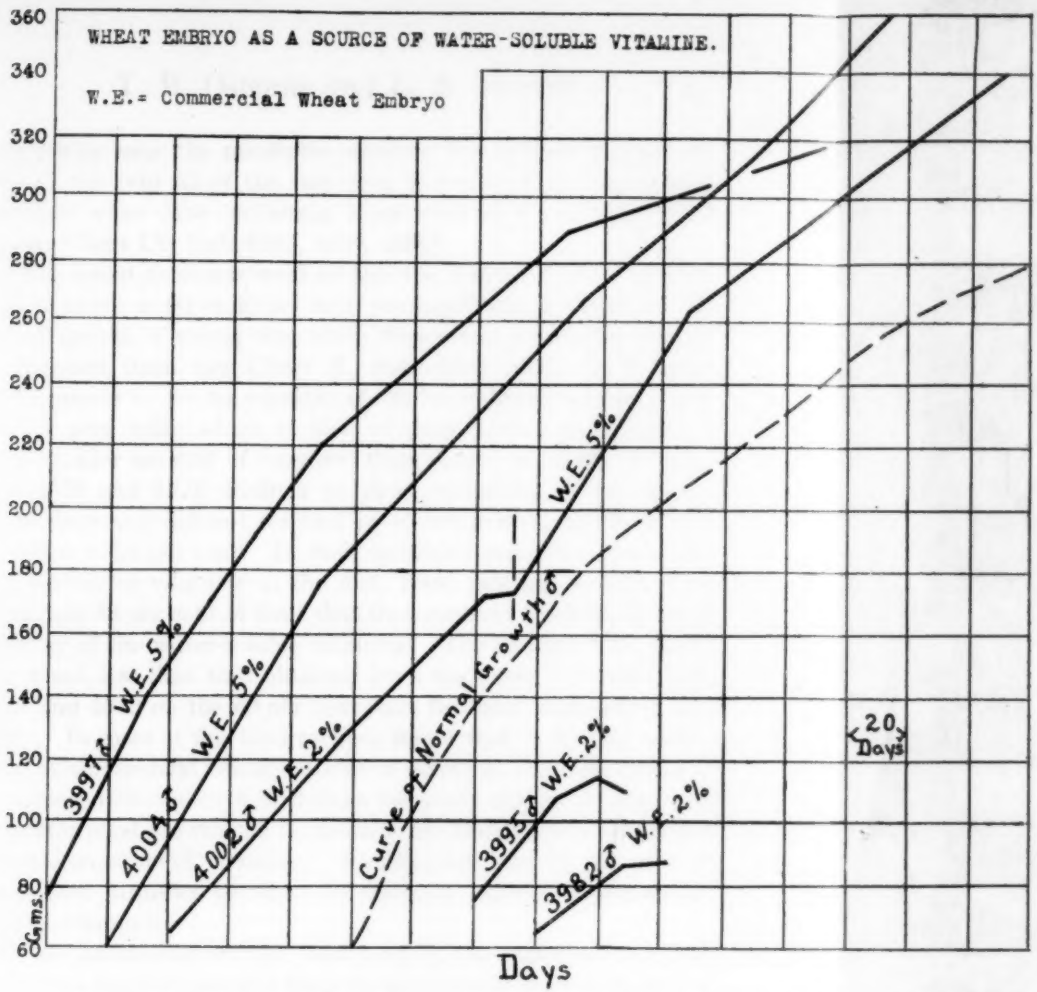


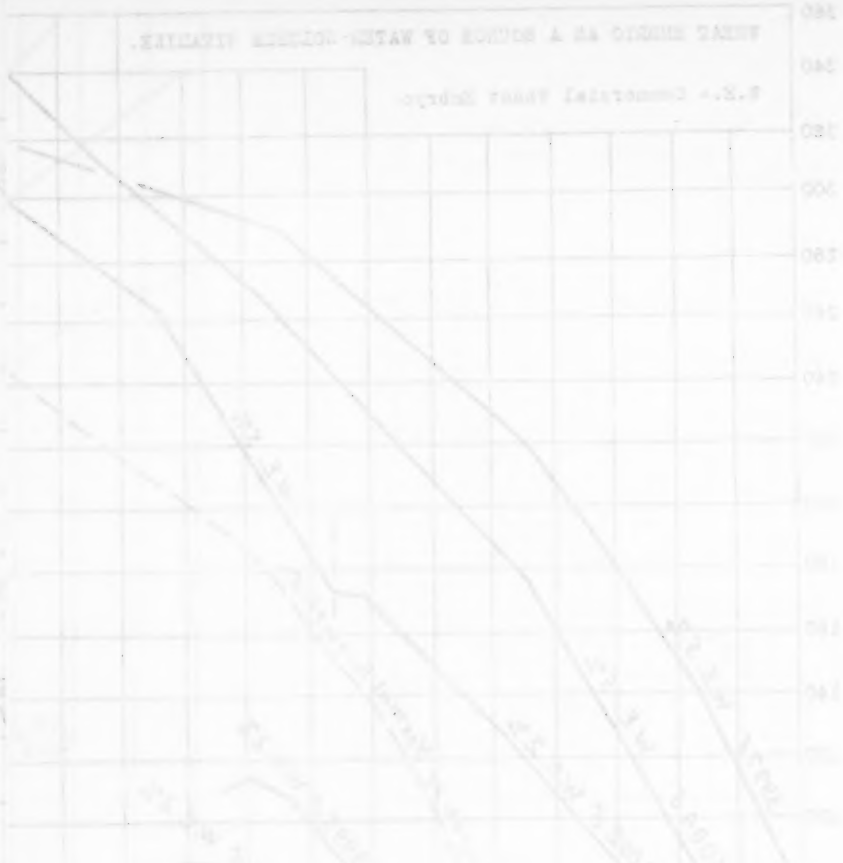
CHART XI. The effect of small proportions (2 to 5 per cent) of commercial *wheat embryo* (W.E.) as the sole source of *water-soluble vitamine* in otherwise adequate diets is demonstrated.

The composition of the food mixtures was as follows:

	Rats 3997, 4004, 4002, Period 2.	Rats 3995, 3982, 4002, Period 1.
	per cent	*per cent*
Casein	17	18
Commercial wheat embryo	5	2
Artificial protein-free milk IV*		29
Salt mixture†	5	
Starch	48	26
Butter fat	18	18
Lard	7	7

* The preparation of this mixture is described in *J. Biol. Chem.*, 1913, xv, 317.

† See legend, Chart I.



is evidently near the minimum required for normal growth, for with 10 per cent all of the rats soon ceased to grow, but always recovered when diets containing 20 per cent of whole wheat were fed (see Chart IX, Rats 4697, 4694, 4699).

When *wheat flour* was used as the sole source of this vitamine, even as much as 50 to 60 per cent was insufficient to promote full normal growth of young rats, while 30 per cent was barely enough to maintain them (see Chart X, Rats 4520, 4517, 4542). Rat 4520 appears to be an example of the exceptional animal, occasionally met with, which is able to grow when supplied with a much smaller amount of vitamine than is needed by the average. Rats 4570 and 4578 declined on diets containing 30 per cent of wheat flour and did not respond by growing when the flour was increased to 58 per cent. In their enfeebled condition due to the low content of vitamine in the diet, these rats ate so little food containing 58 per cent of flour that they received a relatively small quantity of the water-soluble vitamine. The amount was, therefore, much less than that obtained from the outset by Rats 4520, 4517, and 4542 on the 60 per cent food, for these were eating normally. In cases of this kind we have found that by feeding a little yeast or commercial wheat embryo in addition, the food intake is so increased that after 4 or 5 days the yeast can be omitted and thereafter good growth can be made on the former diets containing minimal amounts of vitamine. We conclude that 60 per cent of wheat flour furnishes less than the optimum quantity of the water-soluble vitamine.

Of the *commercial embryo meal* used in our experiments 2 per cent was a smaller quantity than we have found to be adequate to promote normal growth. On an average food intake of 50 gm. per week the minimal quantity would therefore be more than 1 gm. weekly (see Chart XI).

In testing *commercial wheat bran* as a source of water-soluble vitamine it was found that when 5 per cent was used as the sole source of this vitamine in an otherwise adequate ration, *young* rats soon ceased to grow (see Chart XII). *Adult* rats, on the other hand, were not even maintained on similar rations (see Chart XIII). A small amount of commercial embryo fed each day apart from the food mixture containing bran and in addition to it, induced prompt growth of young, or recovery of adult rats (see

Charts XII and XIII). The good effect of the added embryo ceased when the latter was discontinued. Without the added vitamine none of these rats would eat enough food. When 55 per cent of commercial bran, equal to 45.5 per cent of *pure* bran, was fed, as in the experiment where bran was used as the sole source

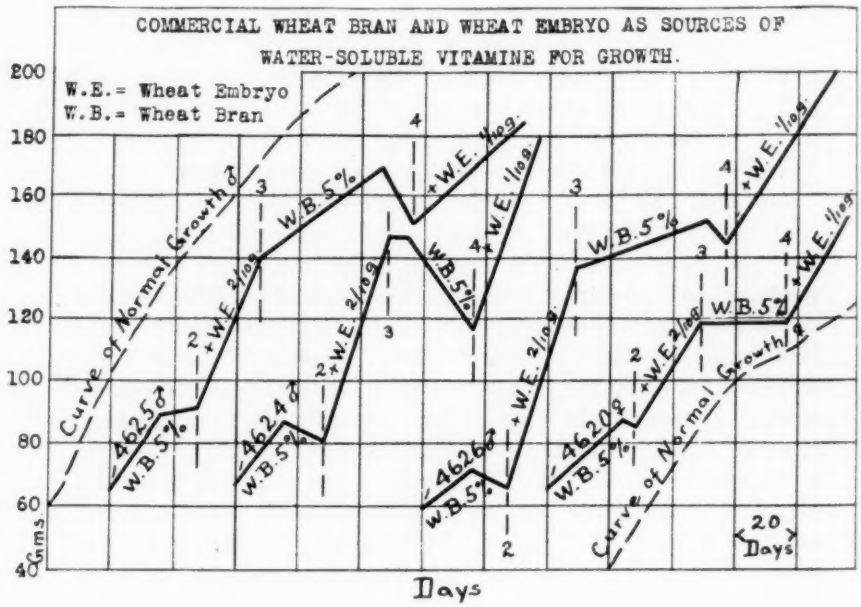


CHART XII. Showing the failure of wheat *bran* (W.B.) to supply enough *water-soluble vitamine*, Periods 1 and 3, in contrast with the rapid gain in weight, in Periods 2 and 4, caused by 0.1 to 0.2 gm. of commercial wheat embryo (W.E.) fed each day separately and in addition to the same diet as was furnished in Period 1.

The composition of the food mixture was as follows:

	per cent
Meat residue*	19
Commercial wheat bran	5
Salt mixture†	4
Starch	48
Butter fat	9
Lard	15

* See Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1917, xxxii, 313.
† See legend; Chart I.

of protein, the addition of wheat embryo had no beneficial effect, showing that the commercial bran used contained some water-soluble vitamine.

Our numerous experiments with commercial wheat embryo meal at first led us to conclude that nearly, if not quite all of the vitamine present in the wheat kernel is contained in the embryo. Comparisons of the quantities of each of the different commercial parts of this seed, however, which, when used alone to supply vitamine, sufficed to promote normal growth, showed that this could not be the case. Accordingly we isolated the embryos from the kernels, taking care to obtain them in the purest possible condition. To our surprise no growth whatever resulted when 22 mg. of the pure embryo were given daily. This amount is equal to

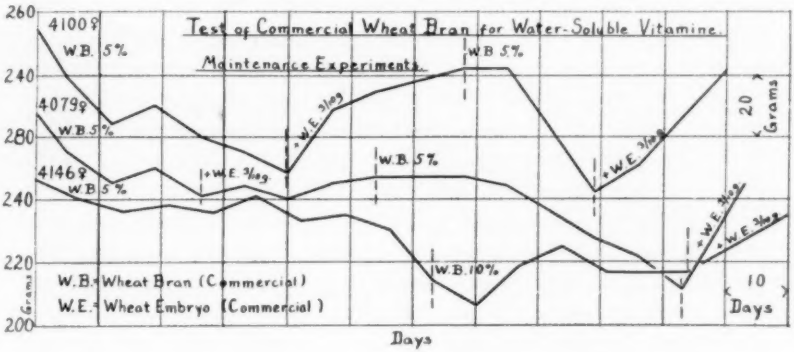


CHART XIII. Adult rats failed to be maintained for any length of time when the *water-soluble vitamine* was furnished solely by 5 per cent of a commercial preparation of wheat *bran* (W.B.). Improvement invariably followed the feeding of small doses of commercial wheat embryo (W.E.).

The composition of the food mixtures was as follows:

	per cent
Meat residue*	19
Wheat bran	5–10
Salt mixture†	4
Starch	48–43
Butter fat	9
Lard	15

* See Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1917, xxxii, 313.

† See legend, Chart I.

the embryo in the 15 per cent of whole wheat used in the experiments shown in Chart IX, assuming an average food intake of 70 gm. per week. The vitamine thus supplied was sufficient to promote practically normal growth. When the pure embryo was given in successively increasing amounts up to 150 mg. the result was the same. Although none of these rats gained appreciably in weight, none of them declined. Thus one rat, which weighed 65 gm. at the beginning of the experiment, weighed only 76 gm. after 71 days, a gain of only 11 gm., instead of 115 gm. which it should have made under normal conditions. Although growth practically failed, maintenance was perfect. Other experiments confirmed this result. On the same diet, with no added source of vitamine, a prompt and rapid loss of weight always occurred.

Since Rats 3997 and 4004 (see Chart XI) when supplied with commercial embryo meal in quantity equal to 180 mg. daily of the pure embryo grew normally, while Rat 4002 which had the equivalent of only 60 mg. daily of pure embryo grew only a little less rapidly, we must conclude that the 150 mg. of the pure isolated embryos given in this experiment should have caused good growth if the water-soluble vitamine of the wheat kernel is a single substance present only in the embryo.

To determine whether the embryo actually contributes anything to the vitamine content of the entire kernel we removed the embryos from a quantity of wheat, taking care to leave as little as possible adhering to the seed, and we believe that at the most only very small traces escaped removal. When these embryo-free kernels were fed as the sole source of water-soluble vitamine they proved quite as efficient in promoting growth as did the entire wheat kernel.

We next cut off about one-quarter of the embryo-free grain at the embryo end of the seed and fed each part separately as a source of vitamine. In comparable quantities the end near the embryo was more efficient than the remainder of the seed though the latter was by no means devoid of activity. Combining pure embryo with either of these parts of the kernel made no apparent difference in the results.

The rats fed on diets in which the pure embryo alone supplied vitamine were well maintained for many weeks without evidence of impaired vigor; but no appreciable growth was made. On the

other hand the remainder of the seed was capable of promoting practically normal growth. These observations raise a question as to whether the water-soluble vitamine is a single substance or a mixture of two or more.

It thus appears that the vitamine is located in the endosperm, but is not uniformly distributed throughout it. This conclusion is not at variance with our experiments with wheat flour or commercial embryo meal nor with Voegtlin, Lake, and Myers' discovery that patent flour is practically free from vitamine. In making flour by gradual reduction the grain is broken into successively smaller particles, a bolting process being interposed between the breaks. The finer particles, which represent the more friable part of the endosperm, are thus sifted out and the larger and harder particles which remain on the sieves are ground into patent flour. If the vitamine is in fact concentrated in the softer parts of the endosperm we should expect the patent flour to be nearly free therefrom and the lower grades to be correspondingly richer therein. The high vitamine activity of the commercial embryo meal may be caused by a considerable proportion of the softer parts of the endosperm adjacent to the embryo which in the milling process is removed together with that part of the seed. Voegtlin, Lake, and Myers say that "the antineuritic vitamine seems to reside in the peripheral layers and the germ of these seeds, whereas the endosperm is relatively poor in this substance."

Our experiments appear to make it certain that if the vitamine is a single substance needed for adequate nutrition, it must therefore be a constituent of the endosperm.

Alleged Toxicity of Wheat.

In a series of papers from the Wisconsin Agricultural Experiment Station[30] upon the effects of rations derived from restricted plant

[30] Hart, E. B., and McCollum, E. V., *J. Biol. Chem.*, 1914, xix, 373. McCollum, E. V., Simmonds, N., and Pitz, W., *ibid.*, 1916, xxv, 105. Hart, E. B., Miller, W. S., and McCollum, E. V., *ibid.*, 1916, xxv, 239. Hart, E. B., McCollum, E. V., Steenbock, H., and Humphrey, G. C., *Proc. Nat. Acad. Sc.*, 1917, iii, 374; *J. Agric. Research*, 1917, x, 175. Hart, E. B., Steenbock, H., and Humphrey, G. C., *Univ. Wisconsin Agric. Exp. Sta., Bull. 287*, 1918.

sources it has repeatedly been alleged that wheat contains a toxic substance. The "inherent toxicity" of the wheat grains is said to reside particularly in the embryo. It is reported that addition of a large amount of wheat embryo frequently produced an early abortion in cattle. Diets high in their wheat content were regarded as particularly injurious to the progeny. The toxicity is stated to assert itself even in the presence of all the recognized factors for growth. In the case of swine,[31] for example, the investigators state:

> "Only in the presence of very liberal quantities of all these factors can the effect of the toxicity be overcome. This toxicity manifests its action by producing important histological changes in the nervous system of the animal, not unlike those recorded for beri-beri. No one important factor for growth, such as better proteins, salts or fat-soluble A, appears able to act as a complete corrective for this toxicity."

Doubts respecting the validity of such conclusions have already been referred to. We have succeeded in growing many rats over a period of a year when whole wheat supplied the protein and water-soluble vitamine. Females have borne litters containing an average number of young (Chart II). Three litters of these have survived, but all were undersized though apparently vigorous. This failure of rats fed on 92 per cent of whole wheat to produce young of normal size is not at variance with the observations of the Wisconsin investigators.

When commercial wheat embryo to the extent of 50 per cent of the diet supplied all of the protein and water-soluble vitamine during nearly a year the rats grew well as a rule and bore several litters of young (Chart IV). Nearly one-half of these young either died or were eaten. This relatively high mortality is, however, not much if any greater than we have encountered in raising rats under experimental conditions. Inasmuch as the surviving young, kept on the same diet, are growing at a nearly normal rate (see Chart XIV) we do not regard the mortality as evidence of toxicity in this diet. The females might have bred more frequently if they had been mated more often.

In view of the successful outcome of these experiments with diets containing such a large proportion of the embryo, in which the

[31] Hart, E. B., Miller, W. S., and McCollum, E. V., *J. Biol. Chem.*, 1916, xxv. 239.

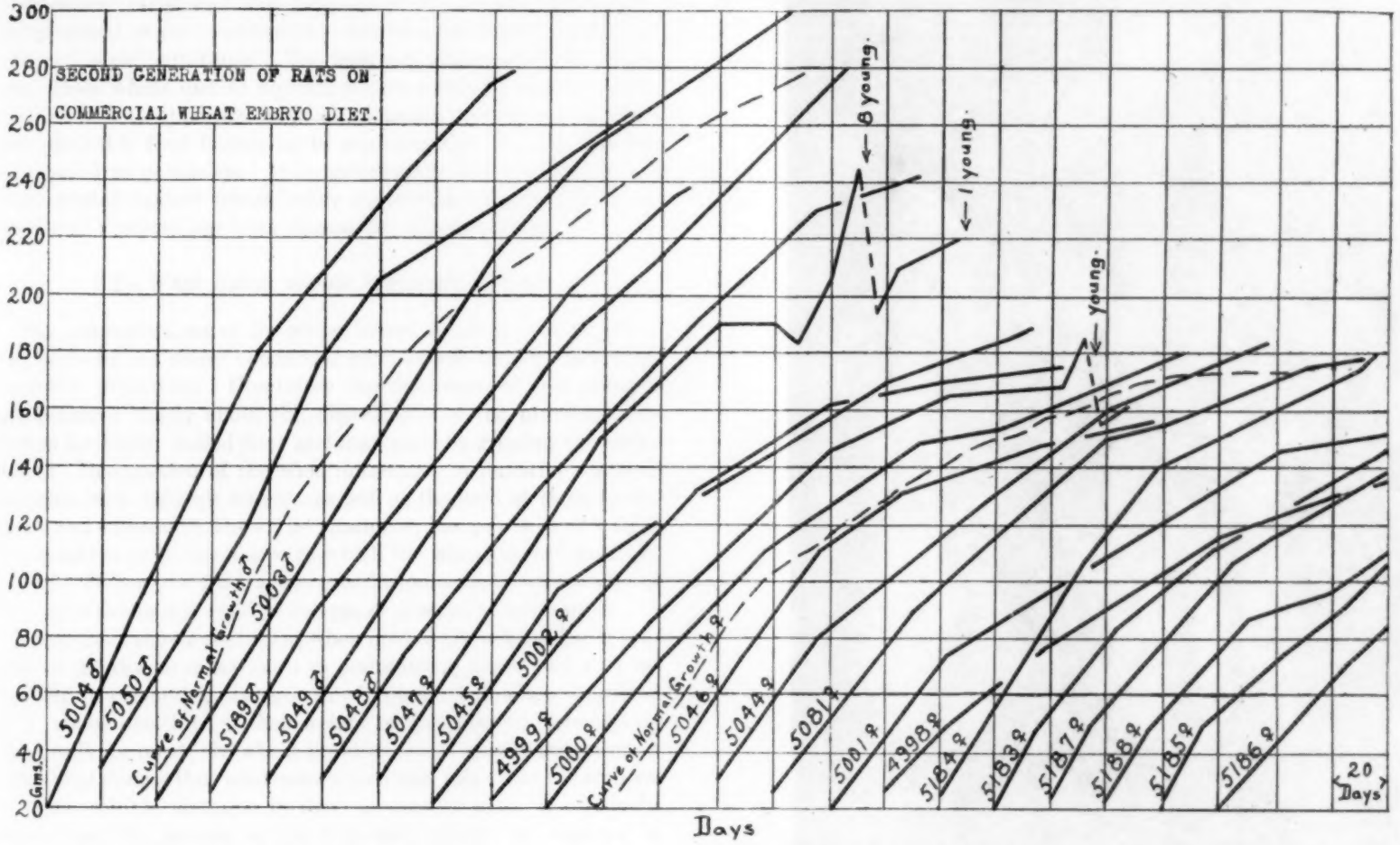


CHART XIV. Growth of the second generation of rats on diets in which the protein and water-soluble vitamine were supplied solely by commercial wheat embryo (50 per cent). The parents of these young were raised on the same diet (see Chart IV and p. 596).

The composition of the food *for both parents and young* was as follows:

	per cent
Wheat embryo	50
Salt mixture*	3
Starch	22
Butter fat	9
Lard	16

* See legend, Chart I.

"poisonous" factor has been assumed to be concentrated, we are not prepared as yet to accept the view that the wheat kernel contains a "toxic" substance. The failure of the rats on the 92 per cent whole wheat diet to reproduce quite normally may be attributed as well to abnormal and inappropriate proportions of otherwise desirable food factors as to anything that may be properly designated as poisonous. It is unfortunate that a prejudice has been created against wheat before convincing evidence of the existence of a poison has been thoroughly substantiated.

To What Extent Should Wheat Be Milled?

The economical use of the wheat kernel demands that its different parts be fed under conditions such as will ensure their most complete utilization. Heretofore the discussion of this problem has centered chiefly about the digestibility of the nutrients furnished by highly milled flour and that made by grinding the entire seed.[32] Recognition of the wide differences in nutritive value of proteins from different sources, as well as the need of those accessory food substances known as vitamines, has put in a new light the question as to the degree to which the wheat kernel should be milled. Before reaching a final conclusion some of the results of the experiments described in this paper deserve consideration.

It has been shown that flour, when used as the sole source of protein, is inferior to other foods in maintaining adult rats, and especially in promoting the growth of young; that when combined with about one-third of their weight of the proteins furnished by eggs, milk, or meat, the wheat proteins are so greatly enhanced in value that flour is thus used most advantageously; that the content of water-soluble vitamine in flour as usually made is relatively small; that the proteins of the bran and embryo are superior in nutritive value to those of the endosperm, from which white flour is made; and that commercial preparations of the embryo contain a relatively large amount of the water-soluble vitamine.

Since by far the greater part of the flour used in this country is eaten in combination with those food products which successfully supplement the nutritive deficiencies of its proteins to a far greater

[32] Various aspects of the question have been presented anew by Snyder, H., *Science*, 1918, xlvii, 429; and Dutcher, R. A., *ibid.*, 1918, xlvii, 228.

extent than do the bran and embryo of the entire kernel, no practical advantage on this score can be expected by converting the entire grain into flour. It is true that commercial embryo meal is rich in water-soluble vitamine and that patent flour is much poorer in this food factor than flour containing all parts of the grain. Nevertheless it is almost certain from what has been learned of the vitamine content of the other food products commonly eaten with bread that the vitamine deficiencies of patent flour are made good thereby. That this is the case is also supported by the fact that the great majority of people who live on the dietaries which prevail in this country show no evidence of ill effects which indicate deficiencies in the amount of vitamines they are receiving.

Furthermore, since the nutrients of bran are poorly utilized by mankind a waste of food, though probably not large, results when the bran is included in the flour. Although the constituents of the embryo have a high nutritive value the embryo forms so small a part of the entire kernel, and so impairs the keeping qualities of the flour, that probably the nutritive advantages of including the embryo in the flour are more than counterbalanced by the practical disadvantages.[33]

We therefore feel justified in the conclusion that, except in special cases, little can be gained by including bran and embryo in the flour when this is used under the conditions prevailing in this country.

When we consider that the rations of farm animals are generally of such a character that protein supplements are needed to increase the proportion, as well as to supplement the chemical deficiencies of their protein, the question arises whether it is more profitable to feed the by-products of milling to animals or to man. The superior supplementing value of the animal proteins produced by the former procedure when used with wheat flour, compared with that of the proteins of bran, as we have shown in this paper, certainly reduces the loss that has heretofore been assumed to result from this practice.

Experience has shown that the proteins of bran are not readily digested by man and that intestinal disturbances are not infre-

[33] Taylor, A. E., War bread, New York, 1918.

quently attributed to the presence of such amounts of bran as are present in flour made by grinding the entire seed. Furthermore agriculturists are convinced that wheat bran is a valuable source of protein in the ration of farm animals. With this conviction the results of our experiments agree.

Armsby[34] has recently demonstrated that coarse fodders, unfit for human food, provide sufficient nutriment for the maintenance of farm animals, and that the energy of the concentrated cereal products supplied in addition thereto is utilized to an extent of 60 to 85 per cent for the production of human food. This view, in conjunction with the fact that animal proteins thus produced, when eaten together with flour, render a smaller quantity capable of supplying the protein requirements of the young, and perhaps also those of adults, further supports Lusk's conclusion, which was based solely on Armsby's data, that "it is evident that food is best conserved for man when edible grains are taken to the miller and the bran is used in meat production."[35]

We can expect, therefore, that the by-products of milling will be better utilized on the farm than on the table. It is difficult to establish what their relative value is when used as human food or fed to farm animals. Many other factors than those of nutrition enter when this question is dealt with in practice; but apart from these, it has been made plain by this investigation, that one has heretofore not received proper consideration. Bran and embryo together form about 17 per cent of the wheat kernel; the endosperm from which flour is made forms the remaining 83 per cent. About one-half as much flour eaten with the animal products, in such proportion that one-third of the protein is furnished by the latter, is capable of satisfying the *protein* requirements as when flour alone furnishes all of the protein. If, therefore, about 80 per cent of the wheat kernel can be so improved in nutritive value by adding animal products to the diet that a much smaller amount of flour will satisfy the protein needs of nutrition, it may well be that the use of the by-products of milling for the production of meat, milk, or eggs will result in a greater economy in the use of flour than if these were used directly for human food.

[34] Armsby, H. P., The conservation of food energy, Philadelphia, 1918.
[35] Lusk, G., *Science*, 1918, xlviii, 447.

To what extent the consumption of wheat flour may be reduced by additions of animal proteins to the ration will depend largely on individual requirements. Adults engaged in hard labor, whose calorific needs are high, can probably meet their demand for protein by the small percentage of relatively poor protein which flour supplies. On the other hand it may be that those of sedentary habits, as well as growing children, can be better and more economically fed when a suitable addition of eggs, milk, or meat is made to the bread ration.

In view of the foregoing considerations the aim of the miller should be to effect such a separation of the other parts of the wheat kernel from the endosperm as will lead to a minimal transfer of the latter into offal. Every grade of flour which is made with a loss of endosperm into milling by-products represents a loss of human nutrients.

Whenever bread made from highly milled flour forms an unduly large proportion of the diet of children the vitamine deficiencies which the experiments described in this paper make so evident, may lead to malnutrition. In this country there is relatively little occasion to be concerned on this score, because, as we have already pointed out, the food habits of our people are such as to make cases of this kind comparatively rare.

That a real danger may be incurred by a too exclusive use of bread made from highly milled wheat is shown by numerous cases referred to by Chick and Hume.[36] They have reported that among groups of people living on restricted diets in which bread made from patent flour formed a large proportion of the total ration, beri-beri was very common; whereas people living on similar diets, but with bread made from the entire kernel replacing that made from patent flour, were rarely affected.

SUMMARY.

The quantity of protein furnished by the *entire* wheat kernel which is necessary for continued maintenance of adult rats is greater than that required when proteins of milk or of various

[36] Chick, H., and Hume, M., *Tr. Soc. Trop. Med. and Hyg.*, 1916-17, x, 141; *Proc. Roy. Soc. London, Series B*, 1917, xc, 44, 60.

other food products are fed. For the normal growth of the young this difference is much more pronounced.

The "crude protein" of the commercial embryo is superior to that of the entire kernel or of the endosperm in maintaining adults. For satisfying the needs of growing rats the embryo proteins are somewhat more efficient than are those of the entire kernel and much more efficient than are those of the endosperm.

The "crude protein" of commercial bran if eaten in sufficient quantity is somewhat superior to that of commercial embryo and decidedly superior to that of the endosperm in promoting the growth of young rats.

For maintaining adults the proteins of the endosperm are adequate; they are inadequate for growth. Additions of meat, milk, or eggs to wheat flour so greatly enhance the value of the protein for growth that a great economy in consumption of protein results. Under the ordinary human dietary conditions the proteins of wheat are advantageously employed.

Commercial wheat embryo is rich in the water-soluble vitamine. Commercial bran and flour contain much less of this important food factor. The *pure embryo*, carefully separated from all the other parts of the seed and used as the sole source of vitamine suffices to maintain young rats, but fails to promote their growth, even when supplied in quantities equal to the amount of pure embryo contained in such quantities of the commercial embryo as were sufficient to promote full normal growth. Wheat kernels from which the embryo has been carefully removed are still rich in water-soluble vitamine.

Rats fed for 1 year from the time of weaning on diets containing 92 per cent of wheat or 50 per cent of commercial wheat embryo reached full maturity without giving any evidence that wheat contains a toxic substance.

The relation of these experiments to the problems raised by modern milling methods and the degree to which it is desirable to mill flour for general use has been discussed.

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